SEX CELL FORMATION IN EXPLANTS OF THE FOETAL HUMAN OVARIAN CORTEX. I

BY

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In most of the cytological, embryological and endocrinological textbooks the *details* of the development of the *human* ovary are described only in rather short terms and often in quite differing ways. However, since the middle of the 19th century a great deal of work has been done on the *cytological* and *histological* development of the mammalian gonad, including some important work on the human gonad, which I should like to consider here.

One of the best descriptions of the cytological development of the female gonad was given by DE WINIWARTER already in 1901.

This author studied a number of serially sectioned human ovaries and compared the results of this work with those obtained by studying a great number of rabbit ovaries. He stated that during the early developmental stages cord-like proliferations were formed, starting from the superficial epithelium of the gonadal region, and he concluded that even the medullary parenchym and the tubules of the rete ovarii originated from the superficial epithelial cells of the celoma. In the ovary of a 7 months old human foetus he was able to find practically the same situation as in a rabbit ovary some 5—10 days after birth, the primitive cortex constituting practically the whole volume of the ovary. In this cortex a great number of ova were found, the youngest always lying just under the superficial epithelium and the more developed ones gradually sinking into the depths of the cortex. The nuclei of the undifferentiated cells of the superficial epithelium mainly had a long shaped form, lying perpendicular to the surface. The nuclear membrane was very thin and the interior of the nuclei showed a fine reticular structure without nucleoli (protobroch A. stage). Going from the periphery into the centre, the nuclei gradually changed their appearance. They became smaller, more ovoid and less stainable, but they still preserved a reticular structure without nucleoli (protobroch B. stage). According to his view, these cells were the future follicle cells. Other nuclei showed a marked growth, became spherical, 1 or

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2 nucleoli developed and the reticular structure became coarser (deutobroch stage). In this way the nucleus of a primary oöcyte was formed and this gradually reached the early prophase of the first meiotic division. Going deeper into the cortex, leptotene, synaptene, pachytene and diplotene stages were found and in the most central parts of the cortex primordial follicles occurred with nuclei in diplotene stages. Apart from these developmental stages, protobroch B. nuclei were found all over the cortex.

DE WINIWARTER also described many symptoms of karyolysis in the cortex ovarii, while mitotic divisions appeared to be extremely rare, because, as he said, in man mitotic divisions in the ovary stopped a long time before birth.

After the seventh month the peripherial nuclei of the cortex reached further meiotic prophase stages, while the nuclei in the follicles showed the resting type (dictuoid stage) which followed the diplotene stage.

DE WINIWARTER deduced from his preparations that, in man, all epithelial cells of the primitive cortex originated from the superficial epithelium and were potentially able to form ova and follicle cells.

In 1930 SWEZY and EVANS also studied the cytological peculiarities of the human ovary.

Summarizing the results of studying the ovaries of a 25 mm human embryo, they wrote: "Here, as in older ovaries, as will be pointed out later, new germ cells arose both by proliferations from the germinal epithelium and by division of already existing germ cells".

According to the ovaries of a 9 cm embryo, they said: "Ova arise from the germinal epithelium by a simple process. Any cell apparently is a potential ovum. The new ovum cannot be distinguished from the surrounding epithelial cells, until both the cell and its nucleus undergo a slight enlargement as the beginning of the process of changing into a germ cell". Moreover a great number of new ova were formed by the division of already existing ones and early maturation of the ova started already in this stage. On this particular point the authors confirmed the findings described by DE WINIWARTER, but between the deutobroch and the leptotene stages they found a spermatocyt-like stage with condensed prochromosomes.

In the 19 cm embryo no more divisions of preexisting ova were found. "Unlike conditions in the two younger embryos new ova in the embryo of this age arise mainly by proliferations from the germinal epithelium".

Starting from the superficial epithelium, a fine stratification of cells in different stages of development was now found, that is, with protobroch, deutobroch, leptotene, synaptene, pachytene, diplotene and some transitional forms between the diplotene and the dictuoid stages.

SWEZY and EVANS did not find real follicles in this period. However, many of the larger ova were encapsulated by connective tissue cells, but "other cells, the so-called indifferent cells, probably epithelial derivates, are also found disposed in the same manner".

According to their studies of the *mature* ovary, the authors mentioned that "it has been found that new sex cells arise in a cyclical manner throughout mature life, the embryonic cells having disappeared sometime between birth and the attainment of sexual maturity".

This conclusion brings us to one of the most discussed points in the study of the development of the gonads viz.: the origin of the sex cells.

A number of contradictory opinions can be found in the literature the vital point always being: do the definitive ova belong to a special line of cells "directly descending in an unmodified condition from the segmenting egg", or not.

With reference to this particular point SIMKENS published in 1928 the results of an extensive study of a relatively large number of human ovaries in different stages of development. Unfortunately this author did not describe the structural details of the ova observed, but he put the problem of sex cell genesis as follows:

Do all the sex cells arise from primordial parents migrating from elsewhere into the gonads, or are the definitive sex cells products of the "germinative" epithelium?

Now in a number of young stages (2, 6 and 7 mm embryos) SIMKENS was not able to find any particular primordial germ cells in the celomic epithelium.

In the 10—11 mm embryo he found cord-like proliferations of the epithelium extending from the superficial layers down into the underlying stroma, and he came to the conclusion that the superficial epithelium is the source of all cells of the incipient ovary [1]).

At the 25 mm stage, the superficial epithelium began to take on the character of an insignificant investing layer, more or less completely separated from the core of the gland.

However, after the 90 mm stage had been reached, he says "after which time no more germ cells or any cellular recrutes from the germinative epithelium reach the epithelial nucleus. Hence from the 90 mm stage onward the histogenesis of the ovary is concerned only with the cells already included within the core". Summarising the results of his own work, the author says: "Shortly after the formation of the epithelial nucleus, which I believe to be the result of a continuous delivery of cells from the germinative epithelium and not from an early and late delivery, two well defined parts can be recognized in the gonad, the nucleus itself and the investing or germinative epithelium; the latter continues to deliver cells

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[1]) In a review on the status of the germ cell problem in vertebrates by EVERETT in 1945, this author summarised the most important and contradictory opinions on this particular point. EVERETT suggested another interpretation of the observed proliferation of germ cells from the germinal epithelium. He said: "It seems probable that the cells of the epithelium, which form functional sex elements, are not and never were a part of the mesothelial covering, but are cells which were segregated early and are merely stored in the epithelium".

into the stroma until the tunica albuginea forms and prevents their ingress; this continues as late as the fifth or sixth month. The primordial germ cells, those delivered from the germinative epithelium during the formation of the indifferent gonad lie deep within the stroma, almost to the hilus, where they are found in greater abundance near the peripherial ends of the rete ovarii. Here they remain until the tunica albuginea is formed and the stroma takes on the character of the cortical and medullary zones. The large cells lie yet within the medullary zone, where, because of their large size, thin chromatin and disunion, they give to that zone its characteristic pale and loose structure. In the medullary zone some of them take on the appearance of follicles, lie loosely associated in nests, and disposed toward the cortex, into which zone they eventually move along with the later transformed indifferent cells, where with the second type of genital cells they enlarge apace, some of them degenerating, others attaining the status of mature follicles at birth. The growth of the primary genital cells into follicles is similar to the secondary genital cells which follows this course; the large cell becomes surrounded by a few small spherical cells, outside of which there are a few small fusiform cells.

The small spherical cells arrange themselves in an orderly manner around the peripheral margin of the cytoplasm of the oöcyt and proliferate to form an irregular double layer. As yet there is no definite cytoplasmic membrane formed around the gonocytes, but its margin can be easily determined by the intense staining reaction of the cytoplasm itself. The irregular disposal of the follicle cells soon passes into an orderly one and soon thereafter the vitelline membrane can be distinguished. The follicles increase in size, so that at birth certain ones have acquired the parts of a mature follicle".

Moreover SIMKENS found between the 5th and 7th month of embryonic development a great number of degenerating follicles in the medullary zone of the cortex and consequently he suggested that the definitive egg cells arise from the more peripherial cells that were delivered to the epithelial nucleus from the germinative epithelium.

Comparing the work of SIMKENS with the above mentioned work of SWEZY and EVANS, it will be clear that the latter do not believe that sex cell formation in the female is limited to the early embryonic period only. On the contrary SWEZY and EVANS suggest the following scheme of the development of the ovaries:

a. an early embryonic period with growth and mitotic divisions of germ cells.
b. a mid-embryonic period with early prophase stages of the first maturation division with a prochromosome stage in the pre-leptotene region.
c. a late-embryonic period with early prophase stages of the first maturation division, without a prochromosome stage and ending in a resting stage (dictuoid stage) shortly after having reached the diplotene stage.

d. a period of degeneration of all ova formed in the embryonic period, from birth to sexual maturity.
e. the period of sexual maturity with a cyclical new formation of ova from the superficial epithelium which shows maturation divisions shortly before or after the ovulation.

In 1932 SIMKENS, contributed to our knowledge of the development of the human ovary from birth to sexual maturity. In the first place he came to the conclusion: "Although I am unable to find any evidence that germ cell division takes place in ovaries older than the sixth month of gestation, except the maturation divisions that take place at ovulation".

His second conclusion can best be summarised by saying that he distinguished two types of "primordial" follicles, viz.:

a. The "primordial" follicle which develops only in embryos and young children. These follicles all disappear through degeneration before sexual maturity has been attained.
b. A second type, the "primary follicle" which occurs in a practically constant number between birth and sexual maturity.

These primary follicles are described as larger, they stain more intensively, are surrounded by at least 1 layer of large round or cuboidal cells and originate in the medullary region of the cortex which means that they are formed in the oldest parts of the ovarian parenchym. The author fixed the number of primary follicles at about 30,000 per ovarium at birth, remaining practically constant until sexual maturity.

Finally the author decided on the existence of solid masses of "granulosa" cells without oögonia. Of these cells he says: "In a position of quietude, indistinguishable from other cells, the potential germ cells remain, probably awaiting stimulation to go forth and produce follicles" and again, ".... the potential masses of cells are always in the ovary, until in very advanced age".

Apart from the work just mentioned it must be remembered that SCHRÖN in 1863 observed an increase of young ova just under the tunica albuginea during the menstrual period in an adult woman. With regard to the follicle cells he discussed the possibility of a mesenchymal genesis. HIS in 1865 indicated the epithelial origin of the follicle cells. KOSTER in 1868 observed growth and formation of young ova and follicle cells in pregnant women of 32 and 37 years of age, as well as in the ovaries of young women (16—17 years old). SLAVJANSKY in 1874 found "Pflüger tubes" originating from the superficial epithelium in the ovary of a woman of 30. FELIX in 1912 did not believe that the superficial epithelium of the adult was able to regenerate new parenchym, because of "the striking absence of mitosis in the germinal epithelium". ALLEN in 1923 again accepted the formation of new ova during sexual maturity. MOMIGLIANO in 1927 stressed the role of the germinal epithelium in the formation of young ova

during embryogenesis, while the follicle cells should originate from indifferent but epithelial cells.

Furthermore, most of the work on ovarian development which can be compared with the foregoing paragraphs has been done on other mammals, although this work is of great importance for the general problems, it indicates however so many differences between the different species that it was thought valuable to restrict our discussions to man alone, since the potentialities of the human tissues especially must be regarded as of decisive importance for a real insight into human physiology and pathology.

Moreover the foregoing already clearly indicates that there are still many problems to solve. Some of these can be summarised as follows:

1. What is the origin of the sex cells. Are there in man, as in many animals, also special primordial sex cells migrating into the gonadal region (ROBERTS) and, if so, are these cells the real progenitors of all sex cells, or do they perhaps induce (NIEUWKOOP in amphibia) the gonadal parenchym in such a way that this tissue (also?) becomes able to form the future ova?
2. If the primordial sex cells migrate into the superficial epithelium, are they always cells of a morphologically different type, or do they behave at least in this respect like their neighbour cells? (DE ROBERTIS; KINGERY).
3. Accepting the superficial epithelium of the ovary as being or becoming potentially able to form new ova (SWEZY and EVANS; MOORE and WANG HSI) is there any time-limit to this potency?
4. Does the power to form new ova still exist during sexual maturity and if so which part of the ovary and which cells must be regarded as the matrix? Does the superficial epithelium possess the exclusive right to produce new ova? (SCHRÖN; KOSTER; ALLEN; ESSENBERGH; OTTO; SCHWARZ; CLAUDE; YOUNG; CROUSE).
5. Do two types of follicles (primordial and primary ones) exist, originating in different periods of gonadogenesis and do primordial and/or primary follicles remain in a position of quietude until sexual maturity has been reached? (SIMKENS).
6. Why are there so many egg cells in the primary stages of the first meiotic division during the latter part of embryonic development and, if these cells are not used during sexual maturity but degenerate between birth and sexual maturity, what can be the meaning of these cells?
7. Are there any differences in life span between oögonia, oöcytes and oötides in different stages of development?
8. Are the embryonic egg cells (especially those which entered the first meiotic division) potentially able to complete this division and can they eventually go into the second maturation division, or are these processes limited to the sex cells during sexual maturity?

9. What is the origin of the follicle cells? Do they arise from the cortical parenchym (HIS; MOMIGLIANO; EVERETT) and/or from the elements of the connective tissue? (NOVAK; SCHRÖN) etc. etc.

Many of these questions were and still are difficult to solve partly because experiments were not possible in human beings. However, since the method of tissue culture has been developed in such a way that experiments on histogenesis and organogenesis have become possible (FELL; GAILLARD), it seemed to be worth attempting the cultivation of fragments of the human ovary in different stages of development.

The material could easily be obtained from the gynaecological and obstetrical clinics and was prepared as soon after death as possible, but in no case after a period lasting longer than 6 hours. The exact techniques of cultivation will be mentioned separately for each of the experimental series.

PRELIMINARY EXPERIMENTS:

In order to study the technical possibilities, a number of preliminary experiments were done between February 1943 and December 1947. The ovaries were obtained from foetuses between about the 16th and 36th week of development and all series will principally be discussed chronologically, because of the technical procedures developing during that time.

Series 18–3–1943. Sixteen weeks [1]) old human foetus.

The left ovary was cut into small fragments ($\pm \frac{1}{2}$ mm^3). 120 fragments were cultivated during a period of 7 days in embryo containers [2]) (see fig. 1).

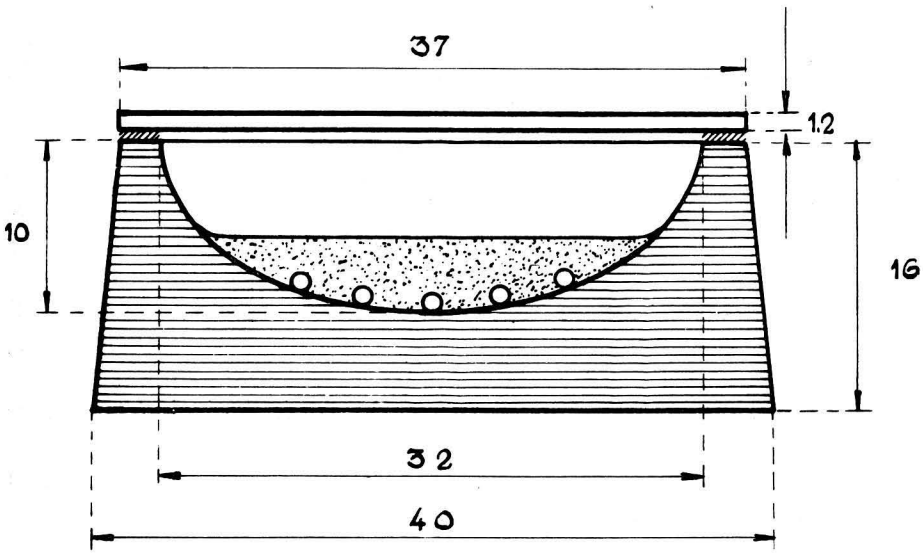

Fig. 1

[1]) It will be obvious that the age of all foetuses can be given only approximately.

[2]) In this and all other experiments the cultures were refreshed every three days.

In each of the containers 30 explants were put into 21 drops of a medium composed of:

10 %	Placental vein serum in Gey's saline solution	10
10 %	Serum of a patient suffering from an agenesis ovarii . .	10
	Chick embryo press juice (9 days)	1

20 Explants were cultivated during 7 days on coverglasses each of them in one drop of a medium composed of:

	Human blood plasma	*aa*	*aa*
10 %	Plac. vein serum		
	Chick embryo press juice (9 days)		

Results: In both the media, even after two days of cultivation [1]), most of the fragments became *encapsulated* by a *cubical epithelium*. At the same time the greater part of the inner mass of the tissue degenerated and after four days of cultivation the aspect of the interior mass had completely changed. *The cell detritus had practically disappeared* and a *mesenchym-like structure was observed instead of it.* Moreover cord-like *epithelial structures* were seen, attached to the covering epithelium and there penetrated into the centres of the explants. No oögonia were found. Unfortunately after six days of cultivation all explants appeared to be completely degenerated.

Series 20–4–'43. Thirty-six weeks old human foetus.

The left ovary was cut into 160 small fragments which were cultivated in embryo containers. In each of the containers 20 explants were cultivated in 10 drops of the following medium

10 %	Placental vein serum in Gey's saline solution	5
10 %	Serum of a patient suffering from an agenesis ovarii . .	10
	Chick embryo press juice (9 days)	1

Results: All explants appeared to be degenerated within two days of cultivation. The non-cultivated control sections showed extensive post mortem degenerations.

Series 17–1–'44. Twenty weeks old human foetus.

The left ovary was cut into small fragments, 75 of them being cultivated for 5 days in embryo containers. In each of five containers 10 ovarian explants were cultivated in 16 drops of a medium composed of:

10 %	Placental vein serum in Gey's saline solution	15
	Chick embryo press juice (9 days)	1

In each of another five containers, 5 ovarian explants were cultivated, together with 5 explants of the anterior hypophysis of the same foetus. The cultivation medium in these containers was made up in the same way.

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[1]) These and all further explants were studied in the living state and after fixation in Bouin-Hollande and staining of the sections with hematoxylin-eosin and Heidenhain "Azan" method.

Results: The ovarian explants in *both* conditions behaved practically alike. *Many explants completely degenerated* within the period of five days.

However *some explants were completely encapsulated* by a cuboidal epithelium. In these explants in comparison with the non cultivated controls *the central mass of tissue was practically unchanged.*

No cord-like "proliferations" were observed. The anterior hypophysis explants were also encapsulated by a cuboidal epithelium. The morphological aspect of the interior mass of this tissue showing the typical structure of the human foetal anterior hypophysis.

Series 25–1–'44. Eighteen weeks old human foetus.

10 small ovarian fragments of the right ovary were cultivated per embryo container in 16 drops of a cultivation medium composed as in series 17-1-'44. 8 containers were used.

In each of 8 other containers, 5 ovarian explants were cultivated, together with 10 explants of the anterior hypophysis of the same foetus.

In 8 further containers, 10 anterior hypophysis explants were cultivated separately. The cultivation period lasted 8 days.

Results: As in the previous series, the hypophysis explants did not influence the ovarian explants.

After 2—4 days of cultivation, most of the ovarian explants were *encapsulated* by a cuboidal epithelium. *In the central parts a severe degeneration* occurred and after 4 days of cultivation the degenerated tissue was replaced by a fibrous connective tissue.

However *at the same time, starting from the encapsulating epithelial layer of cells, cord-like proliferations which often anastomosed were seen to extend into the central mass* (see fig. 1, Plate I).

After 6 days of cultivation, no living ovarian explants were observed; the anterior hypophysis explants behaved as described in the previous series.

Series 6–3–'44. Eighteen weeks old human foetus.

The left ovary was cut into 40 small fragments. All explants were cultivated on coverglasses in a medium composed of:

10 %	Adult human blood plasma (heparinised with 0.5 ml of a 0.1 % solution per 10 ml of blood)	1
	Placental vein serum in Gey's saline solution	1
	Chick embryo press juice (9 days)	one tiny drop

I. 10 ovarian explants were cultivated separately.

II. 5 ovarian explants were each cultivated together with 1 fragment of the foetal suprarenal cortex.

III. 5 ovarian explants were each cultivated together with 1 fragment of foetal suprarenal cortex and 1 fragment of the anterior hypophysis.

IV. 10 ovarian explants were each cultivated together with 1 fragment of the anterior hypophysis of an adult rat.

V. 10 ovarian explants were each cultivated together with 1 fragment of the anterior hypophysis of a new born rat.

Results: Apart from only a few ovarian explants out of group II

which appeared to be encapsulated by a cuboidal epithelium after 2—4 days of cultivation, in all other ovarian fragments a complete degeneration occurred. After 6 days of cultivation no living ovarian explants were found in any of the 5 groups.

Series 11–3–'44. Twenty six weeks old human foetus.

The right ovary was cut into small fragments. All explants were again cultivated in coverglasses and the medium was composed as in series 6-3-'44.

5 Explants were cultivated separately and five other explants were each cultivated together with one small fragment of the foetal suprarenal cortex.

Results: *One explant of the first group and two explants of the second group were encapsulated by a cuboidal epithelial layer* after 5 days of cultivation. The central parts of these fragments had completely died.

No proliferation of the encapsulating epithelium. In the suprarenal cortex fragments the glomerular zones appeared to be in good condition.

Series 20–9–'46. Twenty-eight weeks old human foetus.

The right ovary was cut into relatively large ($1-1\frac{1}{2}$ mm^3) fragments.

In each one of twelve embryo containers, 10 explants were cultivated in 16 drops of a medium composed as in series 17-1-'44.

Results: A bacterial contamination occurred. All explants died within 5 days of cultivation.

Series 30–9–'46. Thirty weeks old human foetus.

From the right ovary 150 small fragments were cut and cultivated in fifteen embryo containers with 16 drops of a medium composed as in series 17-1-'44.

Results: Apart from some follicle cells of the pre-existent primordial follicles, all other tissues showed a complete necrosis within 4 days of cultivation.

No epithelial encapsulation.

Series 2–10–'47. Fourteen weeks old human foetus.

The left ovary was divided into three parts (Two poles and a middle region).

The middle region was cut into slices perpendicular on the axis and from each one of the slices only the most peripheral cortical parts were cut into fragments. Thirty of them *with* a part of the germinal epithelium attached to them and thirty others *without* a part of the germinal epithelium. Embryo containers were used and in each of them 10 explants were cultivated in 15 drops of the following medium:

Gey's saline solution	9
Placental vein serum	1
Streptomycin in Gey's solution	4
	(containing 200 U.)
Chick embryo press juice (9 days)	1

Results: With the exception of one explant belonging to the group prepared with germinal epithelium, all others died within 4 days of cultivation. The "intact" one fixed after nine days of cultivation was entirely encapsulated by a cuboidal epithelium.

However the centre of the explant was fairly necrotic.

Series 16–10–'47. Twenty-three weeks old human foetus.

The left ovary was divided into 3 parts. (Two poles and a middle region).

In this series the *poles* were cut into slices perpendicular to the ovarian axis. From the cortical parts of these slices 50 fragments were cut.

In each of the embryo containers five explants were put *on the top* of a coagulum composed of 7 drops of a mixture of:

Human blood plasma (heparinised with 0.5 ml of a 0.1 % solution per 10 ml of blood)	2
Placental vein serum	1
Streptomycin in Gey's saline solution (containing 100 U.)	2
Chick embryo press juice	2
Gey's saline solution	8

Results: *All explants were surrounded by a flat or cuboidal epithelium* within two days of cultivation. The inner mass of the explants showed at that time an intact cortical structure with a number of deutobroch and leptotene nuclei. Other meiotic prophase stages which were to be seen in the non-cultivated control tissue obviously had disappeared by lysis and other types of degeneration. *After 6 days of cultivation all oöcytes and oögonia had disappeared* and in the cell nest only an indifferent type of epithelial cells remained. After 8 days of cultivation a complete degeneration of the parenchym had occurred.

Series 18–11–'47. Twenty-four weeks old human foetus.

From the poles of both ovaries 140 fragments were cut. In each embryo container 7 explants were cultivated on the top of a coagulum composed of 7 drops of a mixture:

Human blood plasma (heparinised)	2
Placental vein serum	1
Streptomycin in Gey's solution (containing 100 U.)	2
Chick embryo press juice	4
Chick blood plasma	4

Results: Complete degeneration of all explants after 3 days of cultivation. Chick plasma?

Series 21–11–'47. Twenty-six weeks old human foetus (see fig. 2, Plate I).

From the poles of the ovaries small fragments partly *with* and partly *without* the epithelium were cut. In embryo containers 5 explants were cultivated on the top of a coagulum composed of 5 drops of a mixture of:

Human blood plasma (heparinised)	2
Placental vein serum	1
Streptomycin in Gey's solution (containing 10 U.)	1
30 % Chick embryo press juice in Gey's solution	2
Gey's saline solution	4

Results: After 4 days of cultivation *all explants with a part of the germinal epithelium attached to them were encapsulated by a columnar*

epithelium with apical (secretory?) vacuoles. The inner mass of tissue showed some localised degeneration phenomena but between a great number of indifferent epithelial cells *some oöcytes with deutobroch nuclei were still present* (see fig. 3, Plate I).

In the explants without a part of the *epithelium* attached to them *extreme degeneration phenomena* occurred (see fig. 4, Plate I), *the follicle cells appearing to be the most resistent ones.*

After 11 days of cultivation a degeneration of the parenchym was clearly visible also in the encapsulated explants, but a part of the detritus had completely disappeared and in this region, starting *from the surrounding epithelium some cord-like or tubule-like regeneration of indifferent epithelial cells* was to be found (see fig. 5, Plate I).

The explants cultivated *without a part of the germinal epithelium* showed *further degeneration* phenomena (see fig. 6, Plate I).

Conclusions based on the results of the preliminary experiments

1. In cultivating explants of the human ovarian cortex from foetuses of different stages of development (16—36 weeks old) and largely independent of the techniques used, the germinal epithelium appeared to be of great importance. Explants cultivated with a part of the germinal epithelium attached to them always showed the best histological structures and remained alive longer than explants cultivated without the adhering epithelium. Moreover, soon after the beginning of the cultivation period, the epithelium tended to encapsulate the explants with one layer of cuboidal or columnar cells, all having the same morphological appearance. Especially in the last series (21–11–'47) large apical vacuoles were observed in all the cells of the covering epithelium.
2. In three series, cord-like or tubule-like "proliferations" were found in connection with the surrounding epithelium and developing after the inner mass of tissue had been degenerated and replaced by a loose connective or mesenchymal tissue.
   Neither in the surrounding epithelium nor in the parenchymal cords could new formed oögonia or oöcytes be diagnosed.
3. In none of the series could the explants be kept alive longer than about 7—11 days. During the degeneration processes the covering epithelial cells and the follicle cells, which may be present in the central parts of the explants, appeared to be the most resistant.
4. Looking after the different technical procedures used some points can be particularly stressed:
   a. The ovarian poles seemed to be more easily to cultivate than the middle region of the organ.
   b. The cultures cultivated in embryo containers and on the top of a coagulum were the most satisfactory.

c. Heterologous medium components (especially chick plasma) became suspect, because of the extreme degeneration phenomena occurring in the explants.

d. The addition of anterior hypophysis and suprarenal cortex fragments either separately or combined apparently did not influence the behaviour of the ovarian tissues.

DEFINITIVE EXPERIMENTS

After the above mentioned results and suggestions had been obtained, a number of new experiments were done with human ovaries obtained from 4—9 months old foetuses. In these experiments only cortical fragments were cultivated. These were prepared from the poles with a part of the germinal epithelium attached to them. Only embryo containers were used and the explants were cultivated on the top of weak coagula containing only homologous components. According to this, the chick embryo press juice was replaced by human foetal brain press juice[1]) or ascitic fluid and the chick plasma was completely omitted.

The results obtained so far indicated some difference in the behaviour of the younger and older ovaries and, for our present discussion, the experimental series are therefore grouped according to the age of the foetal donores.

A. Results of cultivating ovarian cortical fragments from 14—21 weeks old human foetuses

Series 5–5–1948. Sixteen weeks old human foetus (see fig. 1, Plate II).

The ovarian poles were used for cultivation. 160 explants were cut in such a way that most of them had a part of the covering epithelium attached to them. They were cultivated in 20 embryo containers.

In each of the containers 8 explants were put on the top of a weak coagulum composed of 5 drops (± 0.5 ml) of a mixture of:

Human blood plasma (heparinised)	2	
Placental vein serum	1	
Streptomycin in Gey's saline solution	1	(with 10 U.)
Human foetal brain press juice	2	
Gey's saline solution	4	

Results: After 4 days of cultivation about half the number of explants showed a flat or cuboidal surrounding epithelium.

In the central parts of these explants most of the original parenchym had disappeared and instead of this a loose connective tissue was present.

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[1]) In preparing this press juice the brain tissue was removed from 4—6 months old foetuses aseptically and as soon as possible after death (max. 6 hours). Then the tissue was cut into pieces (diameter ± 1 cm) and placed in a Petri dish at + 4° C. for 24 hours. After that time the tissue was minced with a usual tissue press; an equal quantity of Gey's saline solution was added and the mixture was centrifuged for 15 min. 6—8000 r.m.

The press juice was pipetted in pyrex glass tubes and stored at — 20° C.

Obviously *starting* from the surrounding epithelium a number of *parenchymal cords were observed which often anastomosed* and, *in the cords, numerous oöcytes were observed* (see fig. 2, Plate II).

After 13 days of cultivation all remaining explants were fixed and stained. They appeared to be encapsulated by a *columnar or stratified epithelium from which cord-like structures started* (see fig. 3 and 4, Plate II). *The number of oöcytes had increased considerably,* (see fig. 3 and 5, Plate II) and *leptotene, synaptene* and *pachytene stages of the first maturation division frequently occurred.*

Series 7–6–'48. Twenty weeks old human foetus.

Embryo containers were again used. In each of them 10 explants, cut from the ovarian poles, were cultivated on the top of a coagulum composed as in series 5-5-'48. (Total 200 explants).

Results: Within 4 days of cultivation the explants were *encapsulated* by a cuboidal epithelium grown from the germinal epithelium and in most of the explants the parenchym in the *central parts* had *degenerated* completely (see fig. 6, Plate II).

Some days later the *degenerated mass of tissue* appeared to be partly *replaced* by a very *loose reticular structure* (see fig. 1, Plate III) and at that time a *regeneration* regularly *started from the peripherial epithelium* which could be followed step by step (see fig. 2 and 3, Plate III).

Most of the anastomosing parenchymal cords were *symplasmatic* with numerous nuclei, some of them showing a deutobroch character.

After 10—11 days of cultivation a reticulum of parenchymal cords was obtained and the *number of ova* had *increased* enormously (see fig. 4, Plate III). Deutobroch, leptotene, synaptene, diplotene and early metaphase stages were frequently observed (see fig. 5, 6, 7, 8 and 9, Plate III, and fig. 1, Plate IV).

Moreover, indifferent parenchymal sister cells were seen to envelop the young ova (Epithelial origin of the first layer of follicle cells?) (see fig. 1, Plate IV).

In comparison with the stage of nuclear development, the cytoplasm was generally only poorly developed.

Finally, some of the encapsulating cells showed large apical (secretory?) vacuoles (see fig. 4, Plate III).

Series 2–2–'49. Sixteen weeks old human foetus (170 explants).

In each of the containers 9 explants were cultivated. The medium was composed as in series 5-5-'48, but after 5 days of cultivation the streptomycin was left out and the quantity of blood plasma was halved. Gey's saline solution was added instead of these volumes.

Results: After 5 days of cultivation, a *complete central degeneration* and disappearance of all the remnants of the original structure was observed. From the surrounding epithelium a number of *cord-like pro-*

liferations occurred and in these cords a number of *new oöcytes were found.* After 12 days of cultivation, in two out of five explants *fixed at that time,* a complete filling up of the centres was found by a mass of parenchymal cell cords (see fig. 2, Plate IV). The explants behaved like *miniature ovaries.* In the cords *all stages of the meiotic prophase* were seen (see fig. 2, 3 and 4, Plate IV), *as well as a number of large "primordial" follicles* with their nuclei in the dictuoid stage and with a *well developed cytoplasm* (see fig. 2 and 5, Plate IV). Moreover it became clear that *undifferentiated parenchymal cells tended to envelop the young oöcytes* (see fig. 3 and 4, Plate IV), which again indicates the possibility of a parenchymal origin of the first layer of follicle cells.

Series 8–6–'49. Twenty one weeks old human foetus (150 explants).

The same technical procedures were used as in series 2–2–'49.

Results: The same reactions were observed as usual, viz. the encapsulating epithelium, degeneration of the original tissue and regeneration of cord-like proliferations from the superficial epithelium. It is remarkable that after 6 days of cultivation only very few young ova were seen, but after 12 days of cultivation a number of new oöcytes in early prophase of the first maturation division were observed.

After 15 days of cultivation most of the explants still showed this structure. However, some explants showed another aspect, the centre of the explants being composed of *a mass of collagenous connective tissue,* while *only in the periphery did anastomosing parenchymal cords with oöcytes occur* (see fig. 6, Plate IV). Moreover, these explants were *bordered by an epithelium* of an inverted type, viz. *with the nuclei in the apices of the cells* (see fig. 6, Plate IV). Finally, at some places, a double layered covering epithelium was observed.

Series 25–10–'49. Twenty-one weeks old human foetus.

The explants were made as usual from the polar cortex of the ovaries. Two media were choosen:

A. The same as the one in the foregoing series.

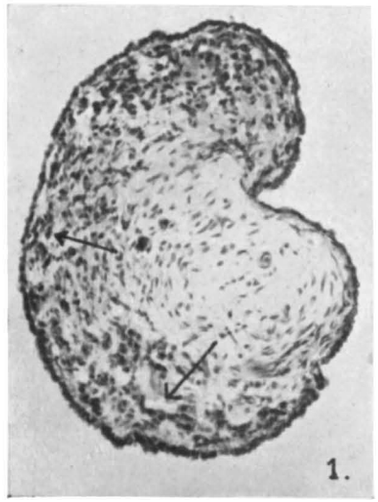
B. A medium in which the foetal human brain press juice was replaced by ascitic fluid from a patient suffering from a carcinosis peritonei, as indicated by C. M. POMERAT (private discussion, July 1949).

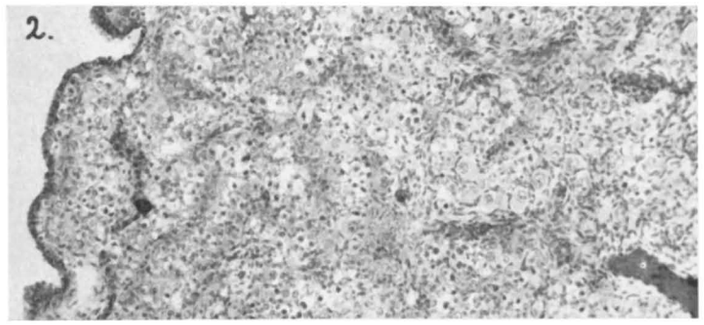
In each of the two media 100 explants were cultivated in embryo containers.

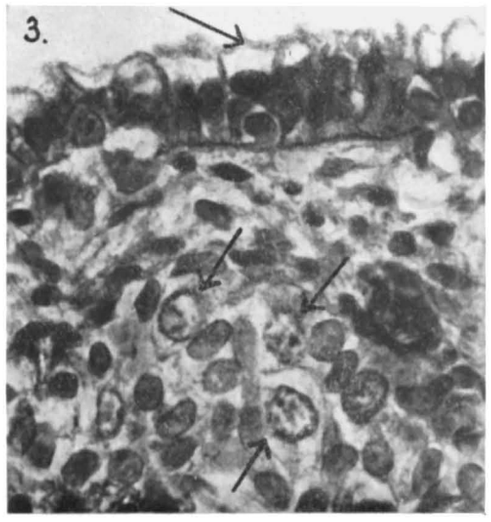
Results: In both media the germinal epithelium encapsulated the fragments as usual. The parenchym in the central parts of the explants degenerated within a few days and a regeneration of cord-like proliferations started from the surrounding epithelium. In these epithelial cords, a number of young oöcytes developed, some of them being enveloped by one layer of cuboidal undifferentiated parenchymal cells.

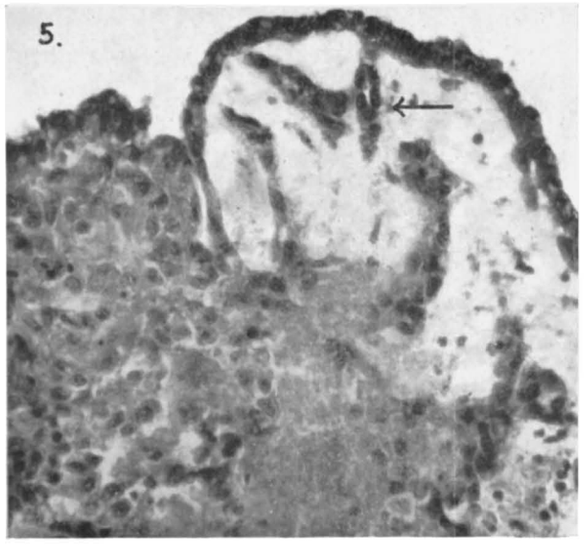
After 17 days of cultivation lumina *in some of the cord-like structures* were seen, which indicated the possibility of tubule formation (see page 1311).

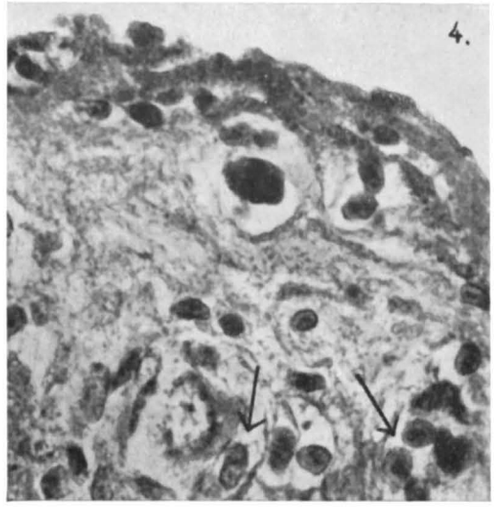
PLATE I











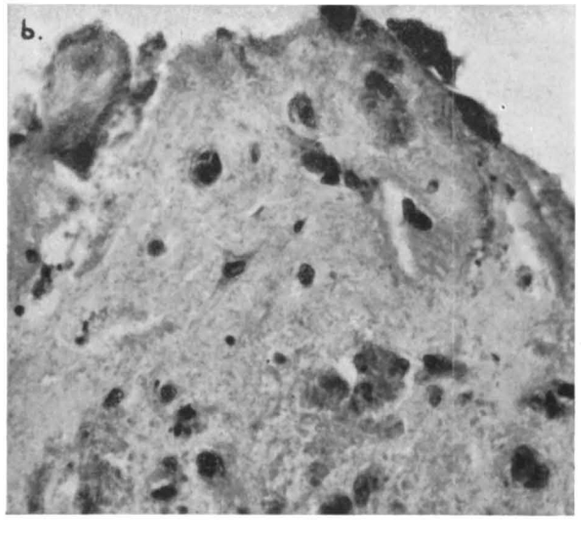
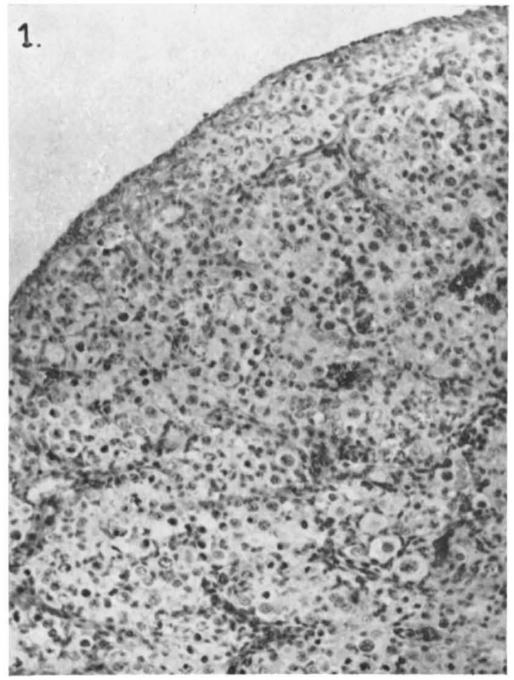
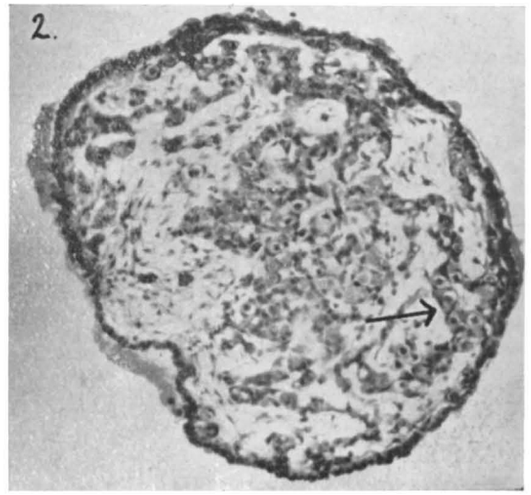
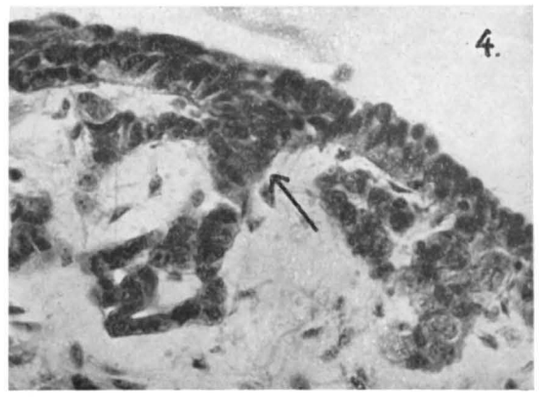
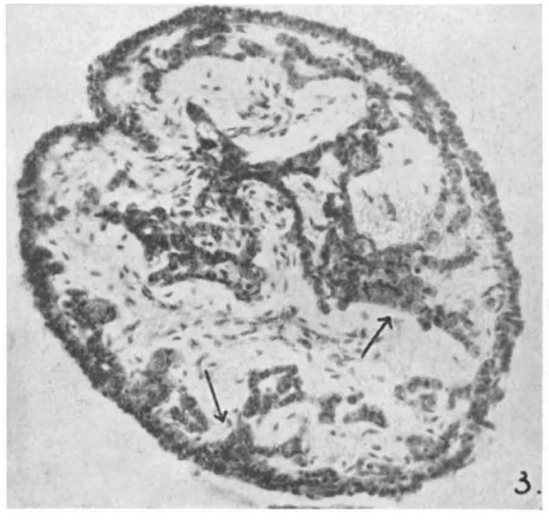


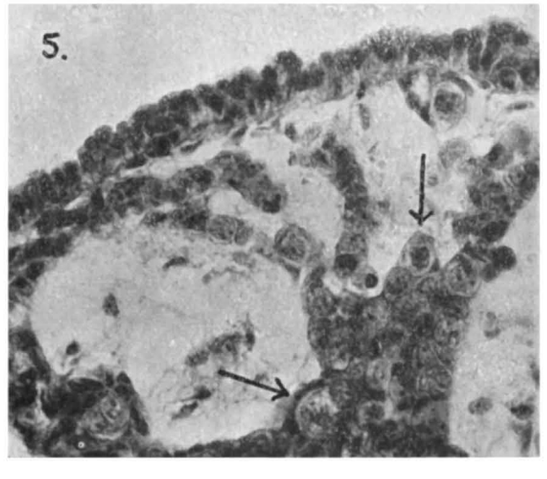
PLATE II











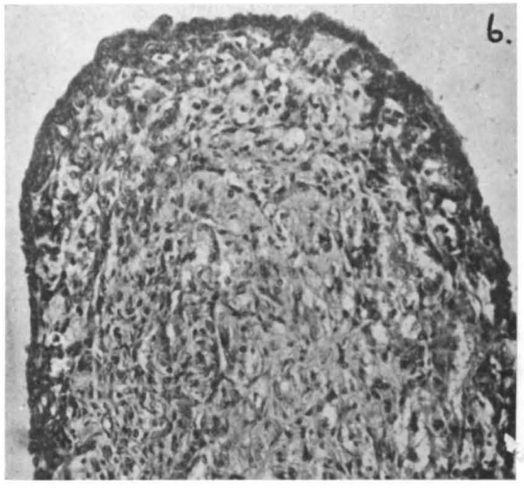
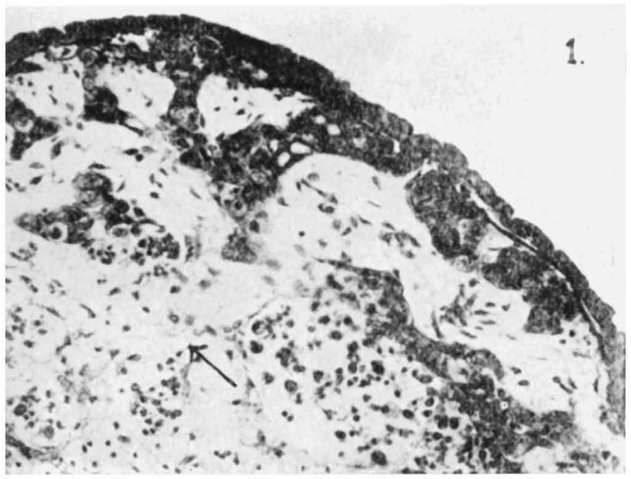
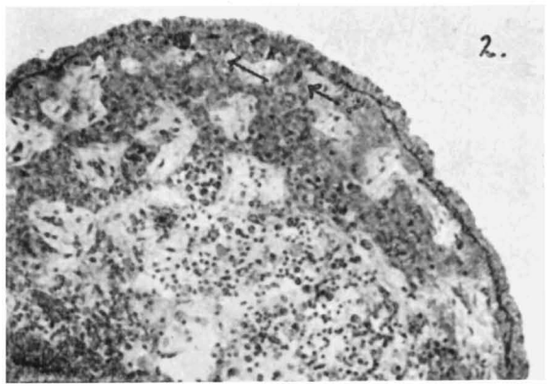
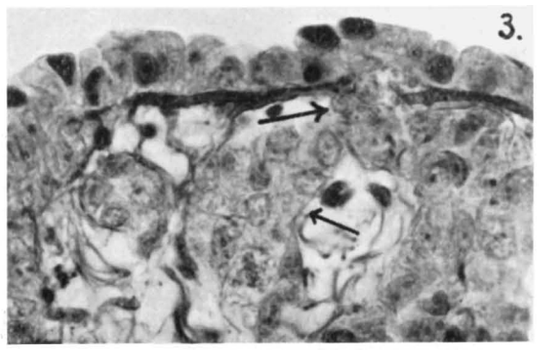
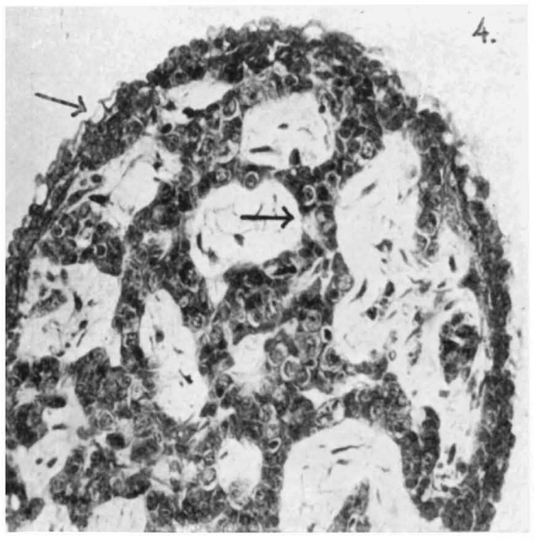


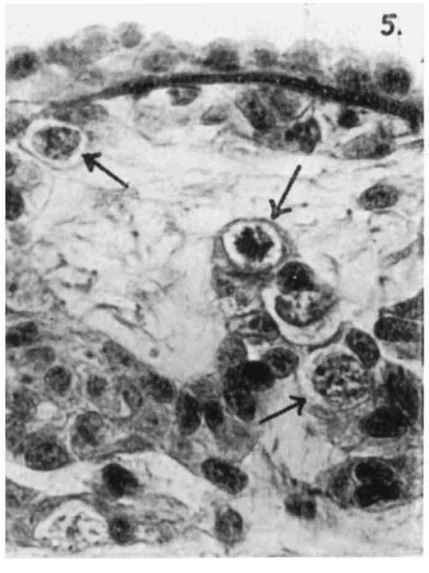
PLATE III

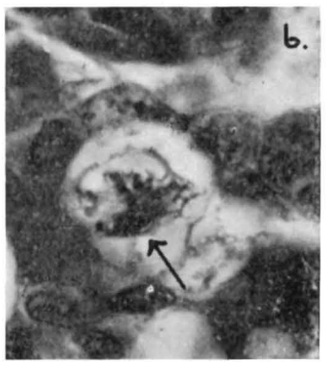


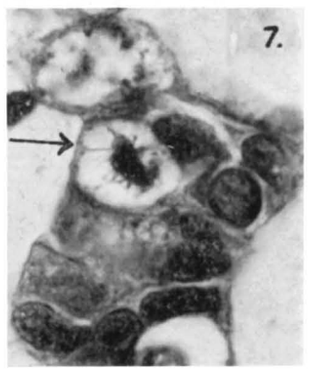


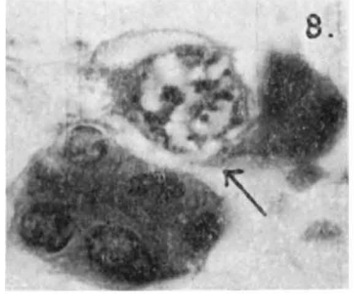












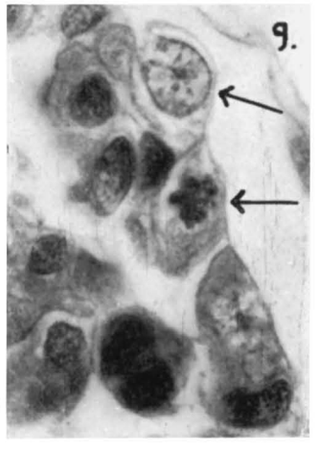
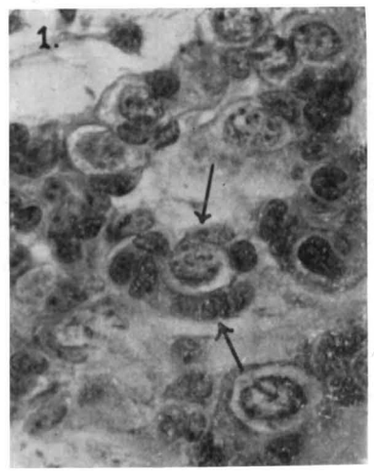
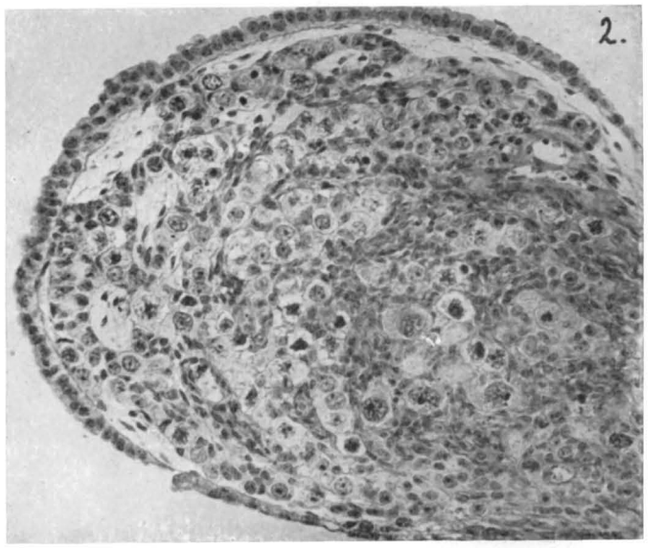
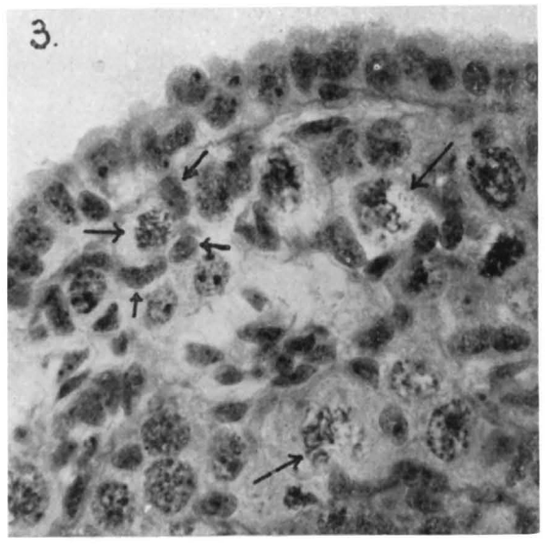
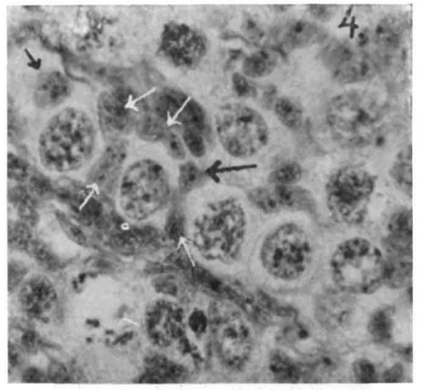


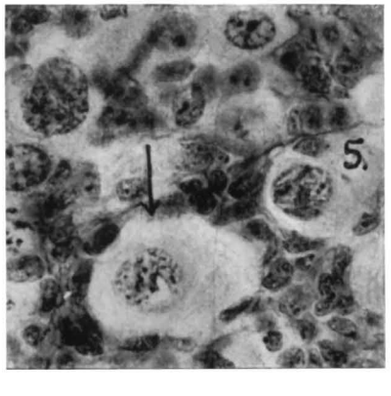
PLATE IV

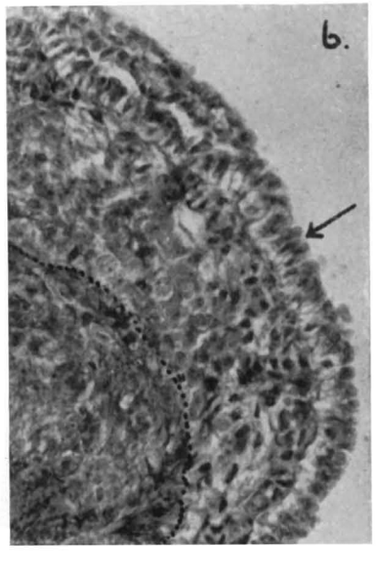


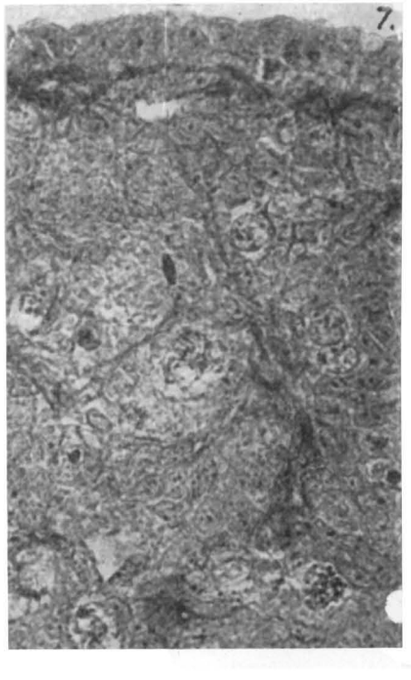












There was no difference of any importance between the explants cultivated in the two media A. and B.

Series 25–11–'49. Sixteen weeks old human foetus.

The same technical procedures were used as in series 25–10–'49.

Results: In this series the behaviour of the explants in the media A. and B. was slightly different. In medium A. exactly the same reactions were observed as were described for the series 25–10–'49. However, after cultivation for 10 days in the medium B (ascitic fluid), many explants had died and only a few survived. These exceptional explants did not show anastomosing parenchymal cords but a very massive parenchym, which was divided into *nests* separated by septa of connective tissue. There were many oöcytes in early prophase stages of the first maturation division (see fig. 7, Plate IV).

Conclusions based on the experiments with explants of the ovarian cortex from the 14—21 *weeks old human foetuses*

It would seem desirable to summarise the results of the experiments so far described with explants from ovaries obtained from the youngest foetuses (14—21 weeks old).

a. In comparison with the results of the preliminary series, the *explants in the homologous media behaved far better* and the cultivation period was extended to 15—17 days.
b. *In all cases the germinal epithelium surrounded the cultivated fragments* by a cuboidal or columnar epithelium. In many of the explants the columnar cells possessed *large apical* (*secretory?*) *vacuoles*, but in one series (8–6–'49) an inversed type of epithelial cells was seen in some explants with the nuclei in the apical parts of the cells and vacuoles in the basal parts.
c. The degeneration of the parenchym of the original explants was regularly observed and *as a rule cord-like parenchymal proliferations penetrated the centres, starting from the superficial epithelium.*
d. The cord-like structures often appeared to possess a symplasmatic structure and *in all series, after* 4—10 *days of cultivation a great number of new oöcytes developed in the regenerated cords.*
e. *In the oöcytes, the first maturation division* frequently started. All prophase stages (leptotene, synaptene, pachytene, diplotene) occurred as in normal embryonic ovaries of this age, viz. with the nuclei in the centres of the cells.
f. In one series, apart from the meiotic prophase stages, an *early metaphase stage was observed*, wich indicates *the possibility of a further progress of meiotic division in the foetal oöcytes.*
g. In two series in the epithelial cords, primordial follicles with one layer of flat or cuboidal follicle cells developed, indicating the *potential parenchymal origin of these cells.*

h. Obviously *the actual stage of development of the egg cell nucleus was not of decisive importance to follicle development*, as follicles were found with sex cell nuclei in deutobroch, leptotene, synaptene, pachytene and dictuoid stages; but the same stages were also found without follicle cells surrounding the oöcytes.

i. Moreover, *the growth of the cytoplasm of the oöcytes appeared to be independant of the nuclear differentiation phenomena.* In most series the cytoplasm of the oöcytes was only poorly developed. However in the series 2–2–'49 a fine vitello genesis occurred and apparently normal, though young, follicles were found with one layer of *flattened* or *cuboidal* epithelial cells enveloping the oöcytes.

j. In one series (2–2–'49) the cord-like parenchymal proliferations increased in such a way that the parenchym practically filled the whole explant. These explants resembled the appearance of an intact but *miniature foetal ovary* with all stages of sex cell formation up to the "primordial" follicles.

k. Finally there were *slight indications of tubule formation* in the parenchymal cords and in the last series there was a suggestion of some difference between the explants cultivated with human foetal brain press juice as a component of the cultivation medium and those cultivated with ascitic fluid.