Anatomy. — "*On a human ovary with a large number of abnormal follicles and the genetic significance of this deviation.*" By M. W. WOERDEMAN. (Communicated by Prof. J. BOEKE).

(Communicated at the meeting of June 26, 1920).

Last year, at the course of microscopic anatomy, sections of a human ovary, which had many peculiarities, were distributed among the students. I made a series of sections of 10 μ of that part of the ovary, that had not yet been cut into sections, in order to make an extensive study of the peculiarities found. After my examination of the preparations and the study of the very extensive literature, I think I am justified in adding another communication to that literature.

The origin of the preparation could not be traced. In the collection of materials of our laboratory it was only mentioned as "human ovary", without any further explanation. As it was not possible to make out, which of my predecessors had added the preparation to the collection, I am ignorant of the age of the individual, from which the organ was taken. It was fixed very well in formaline and was imbedded in paraffine. At a microscopic examination it was evident that there were a great many folliculi vesiculosi of DE GRAAF and a great many atretical follicles. At some places, I think I noticed some luteine cells. This is a sign that ovulation has taken place. Besides, the measurements of the organ in question and the comparison with ovaria of babies make it plausible that the ovary is from a mature individual. But this is only a hypothesis.

For in 1739 already, VALLISNERUS described the presence of ripe vesicles in a newly-born infant and according to E. RUNGE (1906) this phenomenon would appear regularly. The egg-cells would even be fit for fecundation and ovulation would take place. If this were true, the presence of large vesicles and ovulation phenomena would not prove that the individual was mature. But as KÄPPELLI and HEITZ examined more than 200 ovaria of newly-born animals, in which they found large vesicles, but never saw the slightest trace of ovulation, we should not accept without further evidence that ovulation takes place with babies. Whatever the case may be, it is very

probable that the individual, of which the ovary will be described here, is a mature woman. The preparation had several characteristics. First of all, it had a great number of ingrowths of the germinal epithelium, which covers the total organ as a single layer of cubic cells. These ingrowths are small tubes with a cylindrical round lumen, which is surrounded by a small number of bright, cubic cells.

The nuclei of the cells are lying almost in the centre of the cells. All the cells have about the same appearance. Most of the tubes are unbifurcated, only a few bifurcate. They generally do not proceed radially (towards the centre of the ovary), but very often they bend back under the germinal epithelium and proceed more or less parallel to the surface. Consequently one sees many rings of the epithelium under the germinal epithelium (transverse sections of tubes) in the sections. They are lying in the tunica albuginea and end, as far as I could see, blind. In the usual text-books of histology and microscopic anatomy these ingrowths of the germinal epithelium are not mentioned. But, in the text-books of veterinarian histology or those of embryology the occurrence of similar ingrowths in some animals and in human foetuses are mentioned. They are called "Keimschläuche" or "invaginations épithéliales". I will describe them here as "ingrowths of germinal epithelium", because a wrong idea of their genesis adheres to the name of "Keimschläuche".

In the second place the ovary showed a large number of cellular cords within the stroma. These cords are elongated and surrounded by a thin membrane of connective tissue and consist of very bright, regularly arranged, cylindrical cells (see fig. 1*a*). If they had a lumen, they would be exactly like glandular tubes. They have different names in the literature. The most usual one is "Markstränge" (cordons médullaires). Therefore, I will call them in future "medullar cords." The third and most obvious characteristic of the examined preparation is however the presence of a great number of abnormal eggfollicles. In many places, one sees the eggcells lying in groups and surrounded by a number of folliclecells, formed into, what is generally called "eggnests". Besides these eggnests [1]) in which I found as many as 9 eggcells, there were also vesicles (folliculi vesiculosi) with more than one eggcell. In those vesicles a large number of cumuli oophori, instead of a single one, occur (see fig. 1*c* and fig. 3). The greatest number I found was five. But beside the eggcells, which are lying in a real cumulus oophorus, one sometimes finds rudimentary eggcells in the vesicle.

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[1]) Eggball and eggnest may be used alternatively.

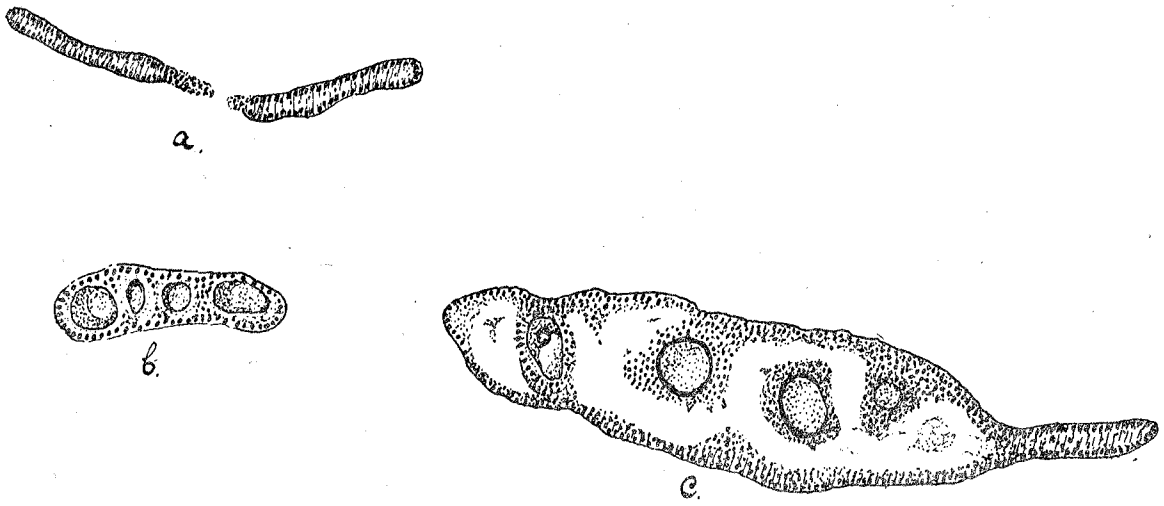

Fig. 1.

a. Medullar cord.
b. So-called eggball follicle.
c. So-called Schlauchfollicle.

Follicles with more than one eggcell are not unknown in man, however. The limit seems to be three. In some animals vesicles are found with numerous eggcells, this is even a normal phenomenon. The human ovary described here is already important owing to the rather large number of eggcells; it gains in importance on tracing the origin of the abnormal vesicles.

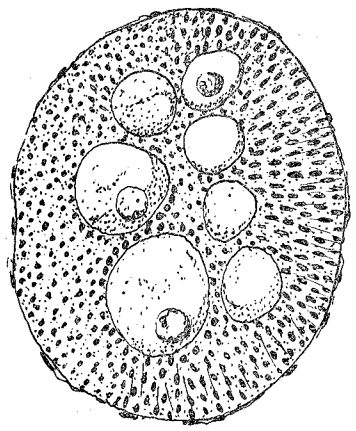

Fig. 2.
So-called Eggballfollicle.

The former conception of the genesis of the ovary was (briefly) the following:

From the germinal epithelium cellular cords penetrate into the ovary (occasionally they have rather the character of tubes or wedges). These so-called "Stränge" or "Schläuche" of VALENTIN PFLÜGER contain the primordial eggs and the future follicle cells. Gradually, connective tissue penetrates through these cords, destroys their connection with the germinal epithelium and divides them into cell-groups.

These cellgroups (Eggballs, WALDEYER) contain a number of eggcells and many follicle cells. Afterwards, every eggcell is surrounded by a single coat of folliclecells and the eggnests divide into a number

of eggcells, covered in this way (which are now called "primary follicles"). The ingrowths of the germinal epithelium are looked

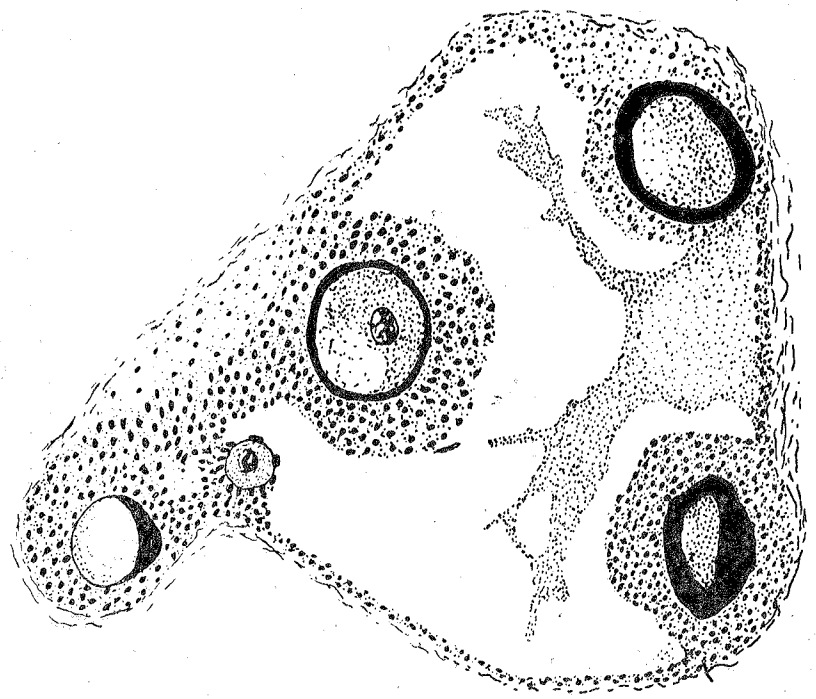

Fig. 3. Atypical follicle.

upon as the rests of tubes of PFLÜGER, originating from the germinal epithelium (SCHMALTZ in ELLENBERGER's Handbuch d. vergl. Mikrosk. Anat. der Haustiere, Bnd. 2, 1911). Consequently the name of "Keimschläuche" is given to those ingrowths. The medullary cords are also looked upon as rests of the proliferations of the germinal epithelium, viz. of the eggtubes of PFLÜGER. If the eggnests have been divided into primary follicles, vesicles, containing more than one eggcell would develop. According to HELLIN (1895) there is a struggle between the connective tissue and the epithelium in the ovary, which generally ends in the victory of the connective tissue. If this is not the case, the division of eggnests into primary follicles, does not, or insufficiently, take place and afterwards a vesicle with more than one eggcell may originate. According to SCHOTTLAENDER (1893) there is a regular relation between the growth of the connective tissue and the germinal epithelium. A disturbance in that regularity is the cause of the origin of the atypical vesicles. In later years, owing to a closer examination, a clearer insight was obtained

into the histiogenesis of the ovary. The excellent researches of COERT (Acad. Dissertation, Leiden 1898) and of VON WINIWARTER (Archives de Biologie, Tome XVII, 1900) may be mentioned here. As not all the embryological textbooks give the same representation of the development, I follow the report, which BÜHLER gives in HERTWIG's Handbuch (1906).

The ovary develops like the testis, from a special part of the posterior coelomic wall, in which the epithelium proliferates and forms a ridge (the so-called Genital-leiste or genital ridge). The line of demarcation between the epithelium of the ridge and the embryonic connective tissue is vague. The sexual cells become visible afterwards in the epithelium. Generally the line of demarcation between epithelium and mesenchyme becomes also clearer. It is irregular. The epithelium penetrates actually with fringe-like ingrowths into the mesenchyme. Those ingrowths are called "epithelial cords" (ALLEN calls them sex-cords). Sexual cells occur in these epithelial cords by the side of undifferentiated epithelium cells. An epithelial proliferation arises from the cranial part of the genital ridge, growing soon inwardly. COERT calls this mass the "reteblastem". When afterwards the genital ridge is tied off more and more, a small body, hanging on the posterior coelomic wall, originates (the undifferentiated sexual gland). There, where it is still connected with the backwall of the coelome, the "reteblastem" lies, from which a number of cords arise, which grow into the direction of the pronephros, as well as towards the centre of the sexual gland (rete cords). The sexual gland consists of a cortical layer, which is nothing but the epithelium of the genital ridge (Str. germinativum or epithelial layer) and an inner mesenchyme mass (Str. medullare). The germinal cords penetrate from the cortical layer into the Str. medullare. Sexual cells occur in the so-called rete-cords and especially in the germinal cords.

From this stage of development differentiation occurs between the development of the male and the female sexual gland.

The convoluted seminiferous tubules arise from the germinal cords, during the development of the testis, the tubules of the rete testis from the rete cords.

During the development of the ovary a thin layer of connective tissue (primary tunica albuginea) is formed between the cortical and the medullary layer. This tunica albuginea lets the epithelial cords pass at many places. A lumen is found, specially in the rete cords, less often in the germinal cords. The rete cords are also connected with the duct of the pronephros. Processes of development occur here, quite homologous to those, taking place in the testis. In the

primary albuginea, germinal cords and rete-blastem, we have to see the homologon of the testis anlage in man.

But in a female body, processes of development take place in the cortical layer besides. Proliferation of the epithelium has taken place here regularly, (in the mean time). The connective tissue of the primary albuginea penetrates into the cortical layer and the cortical epithelium penetrates in many places through the primary albuginea, so that the line of demarcation between cortical layer and medullary layer is again a very vague one. In consequence of the interweaving of the cortical layer and the connective tissue, epithelial cords and epithelial balls develop (not very distinct in man) from which finally the primary follicles arise by further proliferation of the connective tissue.

A complete epithelial layer remains finally at the surface of the ovary (germinal epithelium). The germinal epithelium still forms ingrowths, but they contain very seldom primordial eggs. In any case, they have nothing to do with the ovogenesis and with the eggtubes of PFLÜGER. The name of "Keimschläuche" is in my opinion less desirable. I should prefer the neutral name of "invaginations épithéliales" (VON WINIWARTER). They generally disappear later on. The germinal cords and the rete cords become rudimentary. The germinal cords grow into epithelial cords, lying in the medullary layer. Consequently they are generally called "medullary cords". A number of tubules are left from the rete cords. They are lined with cubical epithelium and lie in the hilus ovarii or even in the mesovarium. Some medullary cords are still connected with the rete. The rete itself may still be attached to the rests of the pronephros (epoophoron).

In the ovary described here (at least in the part I could examine) I did not find rests of the rete, but the ingrowths of the germinal epithelium and the medullary cords were present. With respect to the ingrowths of the germinal epithelium, the following may be said. They are probably a regular phenomenon in the ovaries of human foetuses. After birth they generally seem to disappear soon. A few communications on these ingrowths in infant ovaries are not very clear (cf. SCHOTTLAENDER. Archiv für mikrosk. Anatomie. Bd. 41, 1893). They occur more frequently in young animals and they are even regularly found in adult dogs. (SCHMALTZ). From a figure in the book of SCHMALTZ we may conclude that they are considerably larger in the dog's than in the human ovary, I describe here.

There is a great deal of literature on the medullary cords, which VON WINIWARTER and BÜHLER cite principally. It is evident that they

are observed by many investigators in all kinds of animals during the development of the ovary. They generally break up into primary follicles before birth (COERT, VON WINIWARTER). Then the undifferentiated cells of the cords form the follicular epithelium for the eggcells in the cords. If the medullary cords do not break up into primary follicles before, they certainly do so shortly after birth. BÜHLER could not find them in the rabbit a few days after birth, though COERT and WINIWARTER described them in the embryos of this animal. As VON WINIWARTER found them even 6 weeks after birth in the rabbit, it is evident, that we must take into consideration large individual differences. They seem to appear very regularly in the mature ovaries of carnivores and insectivores. SCHMALTZ mentions them as a regular phenomenon in the dog's ovary and less regular in the cat's.

HARZ, BÜHLER, PALADINO, VAN WINIWARTER, COERT and WICHSER found them in human embryos. RIELANDER (1904) found them in a girl of only a few weeks old. They consist of clear, protoplasmatic cells by a thin, structureless membrane. This agrees remarkably well with what I saw, but is different from what SCHMALTZ saw in the dog's ovary. The latter describes the cords as groups of granular cells with round nuclei, which sometimes surround a small lumen. In newly-born infants egg-cells occur besides (BÜHLER). KEIBEL and MALL (Handbuch der Entwickelungsgeschichte d. Menschen 1911) mention that the medullary cords are rather often found in the first years of life, but only seldom in the ovary of adult women. BÜHLER saw them in a girl of 2 years old, but not in older ovaries. The preparation described here is interesting, because it contains very clearly embryonic rests (ingrowths of germinal epithelium and medullary cords). The appearance of these rests is not so rare that it would justify this communication. I think, however, I can point out a connection between the presence of the medullary cords and the appearance of the numerous atypical vesicles.

While studying the sections of a medullary cord in the series (fig. 4) one perceives that egg-cells still occur in the medullary cords (cf. section 4 in fig. 4 and fig. 1*b*), but one can see at the same time that the medullary cord is able to swell at a certain spot and is transformed there into a vesicle, in which often more than one egg-cell occur.

It is obvious in the series that the medullary cord, after swelling and developing into a vesicle, afterwards regains its former appearance. This is an indication that the medullary cord of fig. 4 is not one that is accidentally connected with a vesicle, but that the

vesicle (Graafian follicle) is a modified part of the medullary cord.

In fig. 1*c* we have a beautiful example of a vesicle, which is

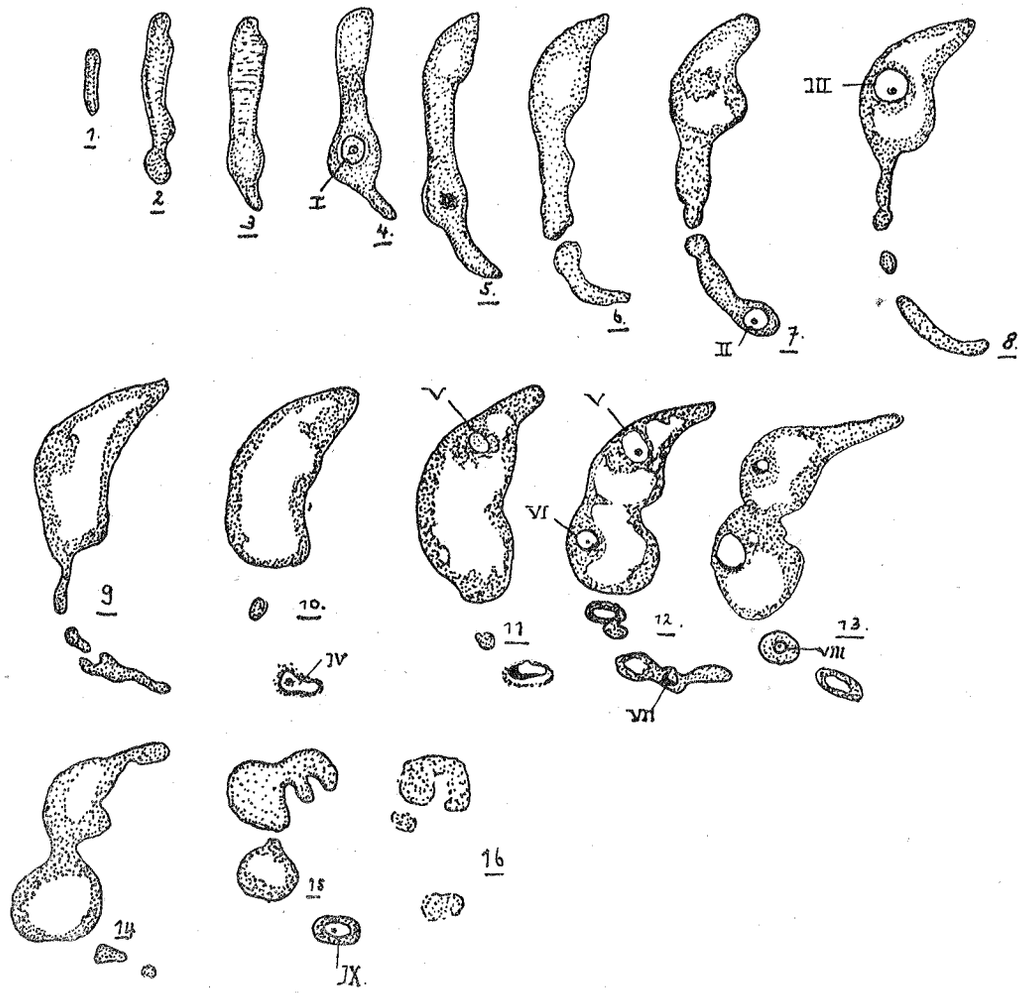

Fig. 4. 16 sections from a series of 80 (each of 10 μ).

merely a swollen part of a medullary cord. A rest of this cord is still seen attached to the vesicle. Though less clear the vesicle of fig. 3 shows such a rest. By these observations, I am convinced that a great number of atypical vesicles in the preparation, described here, originated owing to a proliferation of the epithelium in parts of the medullary cords. Afterwards those cells are vacuolised and a vesicle is formed. The remaining cells form a cumulus oophorus round the egg-cells, which originally occurred in the modified part of the medullary cord. As there are also groups of egg-cells, as reproduced in fig. 2, which must be looked upon as egg-balls, it is

not possible that all the atypical vesicles arise from the medullary cords but that also a great number originates in such egg-balls. I said already before that egg-cells are found in the medullary cords in embryos and also in newly-born infants. The fact that the medullary cords and the egg-nests originate from the same epithelium, explains this phenomenon sufficiently [1]). But all the investigators have found that those cords afterwards break up into primary follicles.

SCHMALTZ calls the eggcells, occurring in the medullary cords, strayed (verirrte) elements and supposes that they are reduced afterwards. In the case of dog, cat and other animals, in which the medullary cords remain, SCHMALTZ does not mention the occurrence of eggcells in those cords or their metamorphosis into vesicles.

The vesicle formation described before, I did not find mentioned anywhere, not even in the text-books of pathological anatomy.

Probably SCHOTTLAENDER (Archiv. f. mikrosk. Anatomie, 1893) found the same thing in man, as described before, but he explains them differently. There is only one text-book of histology (the antiquated book by BÖHM and DAVIDOFF) which, according to the representation of SCHOTTLAENDER, tells something more of the atypical vesicles than the other text-books I consulted.

SCHOTTLAENDER distinguishes "Eiballenfollikel" and "Schlauchfollikel". He calls the follicles, reproduced in fig. 2 and 3 eggballfollicles and those reproduced in fig. 1*c* and fig. 4 "Schlauchfollikel". According to him the origin of eggballfollicles is due to the fact that the eggballs are not broken up into primary follicles, owing to insufficient development of the connective tissue.

He thinks that the "Schlauchfollikel" develop from the tubes of PFLÜGER made free. (In his opinion the "Pflügersche Schläuche" are flattened and elongated eggballs). Undoubtedly SCHOTTLAENDER has seen in the human ovary vesicles, which were more or less modified cords and he described about the same phenomenon I saw. In the atypical follicles of fig. 4 and 1*c* however, I cannot see tubes of PFLÜGER, made free, partly because they do not occur in man according to BÜHLER, KEIBEL and MALL) and partly, because the investigation has taught us, that they are parts of epithelial strands, which undoubtedly must be looked upon as medullary cords.

Like SCHOTTLAENDER, I should prefer to distinguish two types of atypical vesicles: viz. "ballfollicles" and "cordfollicles". Probably, they both originate, owing to an insufficient anlage and development of the ovarian connective tissue; the first because the normal rupture of eggnests into primary follicles failed to take place, and the last, because no primary follicles developed from the medullary cords. If this be true, the two kinds have also a different phylogenetic significance. The cord follicles arise from that part of the ovary which ought to be considered as the rudiments of the male part of the original hermaphroditic, sexual gland (Cf. KEIBEL and MALL, Handbuch d. Entwicklungsgesch. d. Menschen, part I, fig. 8). The ballfollicles are developed from the female part. Though the sexual cells in the rudimentary male part (the medullary cords) undergo exactly the same changes as the eggcells and afterwards actually lie in the real "vesicles", it is yet possible that they are different from those developing in the female part of the sexual gland, (though this is of course not necessary).

[1]) It must not be left unmentioned that VAN DEN BROEK (1895) thinks there is a connection between the medullary cords and the mesonephros and GIANNELLI (1915) thinks they originate in the stroma ovarii.

One might imagine that they will never develop into eggcells, fit for fecundation, and that the vesicles, containing such cells, become atretical.

The follicles in the dog have generally more than one eggcell, but according to SCHMALTZ the larger vesicles contain as a rule only one eggcell, and consequently SCHMALTZ says the "mehreiigen Follikel" seem to disappear. According to BONNET (Lehrb. d. Entwicklungsgeschichte, 1918) in multiparous animals, two or more eggcells are discharged at the ovulation. BUMM (Grundrisz der Geburtshilfe, 12th Ed. p. 292), mentioning that STRASSMANN found in a human ovary two eggcells in nearly all the follicles, and even in the ovary of a woman, who died, while giving birth to twins, writes: "Da man bei Frauen, die nach Zwillingsgeburt starben oft nur *ein* corpus luteum nachweisen konnte, scheint die Entstehung der Zwillingsgravidität aus zwei Eiern *eines* Follikels nicht einmal ein besonders seltener Modus zu sein". Also, according to KEIBEL and MALL, vesicles with more than one eggcell may be the cause of twin gravidity. So it is very probable, that a number of follicles, with more than one eggcell, come to maturity and ovulation. But I wonder, are these not eggnest follicles?

The following consideration led me to this conclusion: In an ovary of an infant of 4 months old, of which I examined a series of sections, I found a great number of rather large folliculi vesiculosi and only some rests of medullary cords. Medullary cords are only seldom found in the calf (MAC LEOD), on the other hand HEITZ and KÄPPELLI found that shortly after birth, and even a short time before, a large number of well-developed Graafian follicles occur. This led me to the supposition whether these follicles, already

present at birth, could not have originated from the medullary cords. Afterwards it appeared that this idea was not a new one. SCHOTTLAENDER already is of the opinion, that most of the Graafian follicles of infants are eggnest- or Schlauch follicles, that is to say, follicles I described before as ball- and cordfollicles.

I recapitulate: BUNGE accepts that the Graafian follicles, present at birth in man, become mature and that ovulation takes place already in infants. On the other hand HEITZ and KÄPPELLI never saw any trace of ovulation in 200 ovaria of newly-born animals, in which they saw however many very large follicles [1]). I think the wisest is to accept (for the present) that the large Graafian follicles present at birth, do not develop, but are absorbed.

According to this idea, the follicles, with an abnormal number of eggcells, in adults, would not be cordfollicles, but ballfollicles. And these would be important for the origin of plurigravidity. We ought to point out besides that several authors talk of "Hauptei" and "Nebenei". That is to say that in a follicle with more than one eggcell one of them were to develop well and the others were to be reduced.

As rudimentary eggcells were often present in the atypical Graafian follicles observed by me, the possibility is not excluded that, owing to reduction of a number of eggcells from the atypical follicles, normal ones finally originate.

In a few words I will answer the interesting question whether a formation of new eggcells takes place in the medullary cords. I did not find anything that points to this fact. I can only say that I found in the ovary of a child of 4 months, which did not show any pathological deviation, primordial eggs in the germinal epithelium and also proliferations of this epithelium in which primordial eggs occur. This corroborates WALDEYER's opinion and that of other investigators, that the formation of primary follicles still continues after birth, an opinion, which according to CUNNINGHAM and ROBINSON is based on observations in pathological cases.

SUMMARY.

1. In a probably adult human ovary were found: *a.* ingrowths of the germinal epithelium, which do not contain eggcells (the name of Keimschläuche is less preferable) *b.* medullary cords which contain eggcells and at some places swell and develop into vesicles, *c.* a great number of follicles with more than one eggcell.

2. The atypical follicles are of twofold nature: there are ball- and cord follicles.

3. In the normal development of the ovary, owing to proliferation of the connective tissue, the eggballs and medullary cords divide into primary follicles, shortly before or soon after birth. In the preparation, described before, the development of the connective tissue was apparently insufficient.

4. It is possible that the large follicles occurring in old foetusus and in infants arise from the medullary cords. This is possible, because larger parts of the medullary cords develop into vesicles, but these may also originate from the primary follicles, which in normal circumstances originate from the medullary cords.

It is doubtful whether the opinion of RUNGE (that these vesicles contain eggcells fit for fecundation) is right, taking into consideration the genesis (medullary cord is homologous with the seminiferous tubules) and considering all we know on this subject in animals. The formation of vesicles from the medullary cords in the above-mentioned preparation, points to a disturbance in the development (a process, normally taking place at a very early age, found in an adult).

5. Normal vesicles may be developed from atypical ones by reduction of all the cells (Nebeneier) except one (Hauptei).

6. It is impossible to state whether the eggball or the cord follicle, or both can give rise to plurigravidity. In this case one ought to know whether the cord follicles are actually ripening to maturity.

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[1]) In a series of sections of the ovary of a young porpoise (Phocaena communis) I found only primary follicles. Clear medullary cords were absent, neither did I see secondary follicles. It is an open question whether 1. probably weakly developed medullary cords have been present, which do not give rise to vesicles. shortly before or after birth or 2 the follicles formed at birth, have all been reduced. I did not find traces of that reduction.