THE HUMAN OVUM
CONCEPTS ON CONCEPTION

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I am deeply honoured in being invited to talk to you this evening. The gynaecologist's knowledge of Anatomy is decidedly limited and I therefore admire greatly the courage of your Society's Committee in directing their invitation to me. However, confined as is the common ground of the anatomist and the gynaecologist, therein lies the focal point of humanity: the human ovum and the process of fertilization.

In this age of population explosion, contraceptive utopia and abortion massacre, the fundamental importance of studying the human ovum and of understanding the fertilization process is acquiring concrete recognition. The application of biochemical, embryological and genetic techniques to the problem of conception has resulted in rapid advances over the past 15 years, in the basic knowledge of fertilization and of very early embryonic development.

The process of fertilization implies a continuum of events. The formation of the ovum, for instance, is becoming better understood. It has been known that in the human all the oocytes are formed before birth. It is now believed that there is a so-called "production line" of eggs in the ovary: those formed early in the foetal stage are ovulated early after puberty, and so on in the woman's life. An increasing proportion of the eggs formed late in the foetus are abnormal; their release is common in the later years of reproductive life, and this may account for the higher incidence of mongolism and other trisomic conditions in babies born to older mothers.

The close study of the human ovary has gained great impetus. Specimens can be obtained at operation, either when ovaries are removed in their entirety in the course of a gynaecological operation or else as a result of partial resection of one or both ovaries for a direct or an associated indication. Besides these surgical procedures, there are two endoscopes which, used in the course of clinical investigation, have enabled the acquisition of specimens of human ovaries without resort to surgical operations: one is the laparoscope, which is an endoscope inserted viâ a trocar piercing the lower abdomen; and the other is the culdoscope, which is introduced through the posterior fornix of the vagina.

In the young adult the ovaries contain many fully-grown oocytes. These primordial eggs have slowly migrated towards the surface of the ovary. The oocyte develops within the Graafian follicle. As cavitation appears in the follicle, the oocyte is fully grown and it halts its progression through meiosis, in diplotene. It may persist for long periods in this phase.

Further development is normally dependent upon the pituitary gonadotrophins. Note that the pituitary is essential not only for the process of ovulation but also in order to trigger off the resumption of meiosis in the oocyte. The surge of luteinizing hormone released by the anterior pituitary in the middle of the menstrual cycle stimulates the further maturation of the ovum within the Graafian follicle up to metaphase of the second meiotic division. It is probable that many human chromosomal anomalies arise at this stage. Normally, however, the ovum rapidly matures into a biologically prepared gamete, with its haploid complement of chromosomes and containing a large amount of recently synthesized ribosomal and soluble RNA.

It is interesting to observe that this difficult phase of ovum maturation has also been achieved in human oocytes under experimental conditions, even without em-

ploying exogenous gonadotrophins. These oocytes can be induced to mature merely by taking them from the follicle and placing them in a suitable culture medium.

Within the Graafian follicle the ovum is set for shedding into the peritoneal cavity. Follicular rupture or ovulation depends upon a fine and specific balance in the release of the two gonadotrophins from the anterior pituitary. It is amazing that this event should occur with such monthly regularity in a woman, considering that the hypothalamo-hypophyseal system is under the continuous influence of a veritable gamut of stimuli and inhibitions. In spite of the involvement of numerous and conflicting neural, hormonal and circulatory factors, a fine balance is achieved in the release of FSH and LH resulting in rupture of the Graafian follicle. Potent preparations of these human gonadotrophins have become abailable for clinical use over the past few years, and their judicious use often results in the successful treatment of anovulatory infertility. On the other hand it is by interfering with the release of the pituitary gonadotrophins that "the Pill" prevents conception: taken daily "the Pill" maintains a relatively high blood-level of oestrogens and gestagen, which in turn inhibits the release of FSH and LH from the anterior pituitary, so that the necessary stimulus to the occurrence of ovulation will be lacking.In our own research experiments at the Medical School, we have investigated the histological changes induced in the entire genital system of female rats as a result of long-term therapy with relatively high doses of intramuscular progestrone depôt: we have been impressed by the fact that, while as expected there was no instance where we detected any ruptured Graafian follicle, yet in spite of prolonged therapy we could still find occasional instances of fully mature follicles with cavitation and well-developed ova, these follicles tending to occur towards the surface of the ovaries.

Some recent studies have been devoted to understanding the mechanics of this follicular rupture at ovulation. Earlier speculation ascribed ovulation to an in-crease in hydrostatic pressure in these follicles; yet recordings have demonstrated that there is actually a fall in intrafollicular pressure at the time of ovulation, so that it is unlikely that rupture is due to such factors as osmosis or muscle-contraction. Recent evidence supports the concept that an enzyme with properties like collagenase is involved in follicular rupture (Rondell, 1970). Such an enzyme doubles the distensibility of strips of follicular wall *in vitro*; while *in vivo* there has been observed a dissociation and reduction in the number of collagen fibrils in the wall of ovulatory follicles, whereas it has been shown that, as in the case of other enzymes, antibiotics are able to suppress ovulation presumably because they inhibit enzyme activity.

At ovulation the follicle bursts and the ovum oozes into the peritoneal cavity. A ripe egg is thus released ready for fertilization, which normally occurs in the ampulla or wide segment of the fallopian tube. To me it is awe-inspiring to see a picture of a human ovum at this stage, and to realize that this indeed is the very beginning of a human being, with all his muscles, and bones and other organs, with his brain and his thoughts, his emotions and aspirations. True it is that the male gamete has its important contribution in this amazing development, but is it equal to that of the ovum? Advances in understanding the process of fertilization, concepts arising from the culture of human oocytes, development of in-vitro insemination and artificial inovulation, novel techniques like nuclear transplantation— all these arouse an increasing array of hypotheses from which, I believe, the theme could be evolved that, in the development of man, the ovum renders an appreciably greater contribution than the spermatozoon. There is more significance than hitherto realized in the fact that the volume of the human egg is 85,000 times that of the sperm.

Let us examine more closely the moment of fertilization. Many spermatozoa approach the ovum; some are stopped by the gummy membrane, the "zona pellucida"; a few break through, but only one penetrates into the centre.

Over the past few years it has been realized that before spermatozoa are able to penetrate the zona pellucida of the egg they must undergo a process of maturation. The spermatozoa stored in the epididymis have completed their meiotic process; and there is recent evidence that in the course of their passage through the epididymis there is an important physiological change in the form of an increase in the degree of adherence of the inner membrane of the acrosome to the subjacent membranes surrounding the nucleus (Jones, 1971). It is believed that an important feature of maturation of spermatozoa in the epididymis is this increased adherence of the acrosome to the nucleus, as it is essential that the adjacent membranes of these two structures remain firmly attached until the very moment of penetration of the ovum.

The final step in the maturation of spermatozoa occurs in the female genital tract itself: it is the process of "capacitation", comprising a number of ultra-structural and biochemical changes which are necessary for sperm penetration of the egg. This conditioning process entails the stay of the spermatozoa for sime time within the genital tract. It may depend upon the fluid environment of the uterus and fallopian tube: in the rabbit, for instance, the uterus is one site where capacitation takes place. In other species, fluid from the Graafian follicles serves to induce capacitation. It is of interest that progesterone has been shown to have a powerful inhibitory effect on sperm capacitation, and that many oral contraceptives containing progestational compounds exert an inhibitory effect on the capacitation of spermatozoa. I wonder, too, whether we should not pay more attention in this regard to the constituents of the seminal fluid, such as fructose, cholesterol and prostaglandins, for they may well exert an influence when the semen is in the female genital tract. It is interesting, for instance, that there is a strong correlation between abnormally low concentrations of seminal prostaglandins and infertility, in both normal and oligospermic men; so that due importance should be accorded to the recent report that the prostaglandin content of human semen was reduced in subjects taking aspirin for one week, thereby suggesting that anti-inflammatory drugs may reduce male fertility (Collier and Flower, 1971).

The ultra-structure of fertilization in mammals has been studied in great detail in recent years, especially with electron microscopy with and without histochemical techniques. The study of spermatozoa has been greatly facilitated by the employment of vital staining with aminoacridines; there is red fluorescence of the acrosome, and green fluorescence of the nucleus.

The acrosome should be considered as a cytoplasmic organelle, that is, outside the nuclear membrane. It is of the nature of a lysosome, containing hydrolytic enzymes within its own membrane. It is a modification of this acrosomal membrane which is probably concerned in the phenomenon of capacitation. In other words, capacitation is a priming of the sperm setting up a so-called "acrosomal reaction", a process which varies in detail in different species. For example, in mammals there is vesciculation and dissolution of the outer acrosomal membrane and the overlying plasma membrane; this allows the release of the acrosomal enzyme hyaluronidase. This enzyme disperses the loosely-arranged cluster of cells surrounding the ovum, the cumulus oophorus, but it does not affect either the corona radiata or the zona pellucida. Passage through the densely-arranged corona cells is facilitated by the bicarbonate ion content of the tubal fluid (a process which is delayed by carbonic anhydrase inhibitors). (Stambaugh et al., 1969; Noriega and Mastroianni, 1969) The spermatozoon then becomes apposed to the zona pellucida, an acellular layer of polysaccharides, which require a trypsin-like enzyme in order to be penetrated. As the acrosome dissolves, a trypsin-like enzyme, which has recently been named "acrozonase", is released either from the region of the inner acrosomal membrane or else (as suggested in very recent studies) from the region surrounding the nucleus posterior to the acrosome (Yanagimochi and Noda, 1970; Teichman and Bernstein, 1971). Fusion occurs rapidly between this post acrosomal

region of capacitated spermatozoa and zona-free eggs, but uncapacitated spermatozoa fail to attach to such eggs.

As dissolution of the acrosome has exposed the sperm head, and as the post-acrosomal region of the sperm head is now fused to the zona pellucida, the continuing activity of proteases digests the zona, which is then pierced by a thickened projection or perforatorium at the anterior end of the nuclear membrane. Then will the sperm nucleus penetrate into the ovum, and the two pronuclei come to lie side by side.

At this moment of fertilization in the fallopian tube three major events are initiated: block to polyspermy, transfer of genetic material and activation of cleavage. This is the very beginning of a new human person.

The block to polyspermy implies some change at the surface of the fertilized ovum which prevents penetration by further spermatozoa. The nature of this block has been studied recently (Conrad et al. 1971) and it seems that after fertilization by the first spermatozoon the cortical granules of the ovum release substances which are able to inhibit the activity of acrozonase, the trypsin-like enzyme from the post-acrosomal zone of the sperm head which is essential for the dissolution of the zona pellucida of the ovum. Thus, further penetration of the zona by spermatozoa is inhibited or blocked by the release of acrozonase-inhibitors. If this mechanism fails, the zygote receives an extra set of chromosomes and becomes triploid; in man triploid embryos die "in utero".

The transfer of genetic material is the second major result of fertilization. It has hitherto been believed that all this hereditary determination lay in the nuclear genes, and that our genetic inheritance was half maternal and half paternal. It is true, of course, that in the zygote half the chromosomes are derived from the female pronucleus and half from the male pronucleus, after fertilization. Yet it is important to realise that the carrier of genetic information, DNA (or deoxyribo-nucleic acid), is not entirely confined to the chromosomes. There is clear evidence that other cell organelles, both inside and out-side the nucleus, also possess DNA and are self-replicating, and that large quantities of extra-nuclear DNA exist in the eggs of many species (including man). In other words, early embryonic development is determined not merely by the the chromosomes from both parents, but also to a very great extent by two other sets of factors which are mostly maternal in origin: intra-nuclear organelles and cytoplasmic or extra-nuclear constituents. It has been shown that ova contain large quantities of DNA, that much of this DNA is stored in cytoplasmic mitochondria, that these mitochondria contain the entire system necessary for protein synthesis, and that cytoplasmic DNA is chemically distinct from the nuclear form and is synthesized and degraded more rapidly (Patton and Villee, 1968). It is emphasized, then, that some of the factors determining the future embryo are laid down during cogenesis, that is well before ovulation and fertilization. Moreover it is now known that in the initial stages of embryonic development no new messenger RNA is synthesized: use is made solely of the maternal "templates" synthesized during oogenesis. During early cleavage protein synthesis is coded for by messenger RNA that had been synthesized in the egg prior to entrance of the sperm.

It appears that our views on heredity have to be modified. We must accept the fact that early embryonic development is essentially determined by maternal factors rather than equally between the parents, and surely it is this very early development which largely shapes the individual human being. Secondly, we will have to understand the significance of cystoplasmic inheritance as distinct from nuclear: it may explain, for instance, the otherwise polygenic inheritance of spina bifida and anencephaly, and the well-known but mystifying correlation between dizygotic twins and neural tube defects (Nance, 1969).

The third major event resulting from fertilization is the initiation of cell division or cleavage. The pre-implantation embryo is an actively dividing and metabolizing organism which changes continuously from the moment of fertilization.

In the human embryo, the early period of pureyl maternal control of mitochondrial activity extends probably up to the 8-cell stage, yet even in this interim period there are profound structural and metabolic changes in the embryo, so that some biochemical characteristics of the very early embryo are determined by events in oogenesis. Later the paternal factors become active as well, and the biochemical features of the embryo alter. It has been shown that the metabolic capabilities of the early embryo change with development; they also reflect the environmental differences as the developing embryo gradually moves along the fallopian tube to the uterine cavity. It is no wonder that certain drugs taken by the mother even at this very early pre-implantation stage are capable of affecting the embryo, in spite of the fact that there is no contiguity of tissues. There is also mounting interest in the events involved in the early stages of cellular differentiation in the embryo. One approach is the study of "clonal development", that is the estimation of the least number of embryonic cells that are involved in the formation of major organ systems. For instance, the precursors for the haempoietic system have been estimated to be fewer than ten cells, though not less than five (Wegmann and Gilman, 1970; Gandini et al., 1968).

Incredible advances are continually being made concerning and affecting the process of fertilization and early embryonic development. There is no doubt that we are at the beginning of a biological revolution which will influence human life far more profoundly than the industrial revolution of the last century or the technological revolution that has overwhelmed our generation. We are witnessing events in this "brave new world" of human biology which formerly would have appeared only in the pages of a science fiction novel. Such indeed is the development of a stainless steel "uterus" which can briefly maintain a human foetus, the intact placenta floating above it, whilst a valve controls the flow of nutrient substances to the fluid bathing the foetus. Oxygen in minimal amounts is supplied similarly, but a major problem remains in getting rid of the foetal waste products.

Remarkable progress continues on several other biological fronts — all of them bound to bear human application. From artificial insemination (which today, for instance, accounts for 10,000 babies born in the United States every year) biologists have advanced to the new technique of "artificial inovulation", a procedure which entails the collection of fertilized eggs from donor animals and their transfer by surgical means into suitably prepared uteri of recipient mothers; several methods have been worked out enabling successful transfer of fertilized eggs and very young embryos. Again, from the success obtained with frozen semen (which is stored at the temperature of either solid carbon dioxide or liquid nitrogen and then remains fertile even after the lapse of 15 years or more), biologists are moving towards success with frozen embryos since unfertilized ova are more difficult: a technique is being developed for freezing fertilized ova which yields a high proportion of viable embryos, a difficulty being that rupture of the embryo may occur either during freezing or during subsequent thawing (Whittingham, 1971).

Another remarkable achievement is that of nuclear transplantation, which has been applied satisfactorily to several species of amphibia though not as yet, for technical reasons, to other vertebrates. The living nucleus from a somatic cell of a tadpole has been transplanted into an unfertilized ovum of a frog, and this egg developed into a normal frog (Gurdon, 1968). There are several points worth emphasizing here: (a) the egg had not been fertilized, in fact its own nucleus is either removed or else killed by irradiation; (b) the donor nucleus is obtained from an epithelial intestinal cell, that is, a cell that is already specialized or differentiated; (c) it was possible to obtain from this combination an entirely normal frog, not simply with intestines (from where the differentiated nucleus was taken) but with all its muscles, nerves, blood cells, and so on. The success of this technique of nuclear transfer shows that in the course of

cell differentiation genes are not lost or permanently inactivated. It also demonstrates clearly our earlier observation that the cytoplasm of the egg has an important controlling effect on nuclear activity; in other words, the cellular components which control gene activity are located in the cytoplasm, and in the fertilized ovum it is this maternal cytoplasm which determine the nature of nuclear activity.

Of all the biological achievements, however, I feel that the most exciting is bound to be the culture of human ova and their "in vitro" fertilization. This entails the recovery of human oocytes (as mentioned at the beginning) and their culture in proper media, which seem to require the presence of cells from the cumulus oophorus as well as pyruvate, serum albumin and other nutrients — but very little oxygen (Kennedy and Donahue, 1969). To these ova in artificial culture must be added the fertile sperm, which however is ineffective unless the problem of capacitation is overcome. All this has been achieved in several centres, and there have been now a few isolated claims for successful fertilization of human ova in culture. Evidence for such an achievement, however, is very difficult to obtain, though it may well have occurred in one or two instances. Nevertheless it seems that complete fertilization with subsequent cleavage has not yet been convincingly accomplished in the human. One can sense a feverish race to be the first in this achievement, yet is the rush not too hasty? The ultimate experiment — culture of follicular ova, fertilization "in vitro", culture up to the morula stage, and placement in a mother's uterus — all this may not be long in being carried out in the human. Yet surely it is not too much to demand that, before such definitive human experimentation become acceptable, a necessary prerequisite is that these studies be carried out extensively in the subhuman primate, with clear evidence that conditions are finally such as to result in the delivery of normal offspring (Mastroianni and Noriega, 1970).

And what are the implications? What fantasy today may become fact tomorrow? It is possible that a married couple may be able to go to an "embryo bank", select a frozen embryo, and then have it implanted in the woman's womb, for subsequent delivery of a baby who carries not their own hereditary features but genetic qualities for which they selected the embryo, qualities of mind, of character, of physical fitness. Less remotely, it should not be long before an infertile couple will be able to have their very own child, employing their own gametes for in-vitro fertilization and artificial inovulation. Much farther away is the development of an efficient artificial womb, in which the human embryo and early foetus matures to a stage where it can then thrive in a modern incubator — but the prospect of such an event recalls the baby factory envisioned by Aldous Huxley 40 years ago. Then, however, although it is true that the baby would thereby be protected from maternal drugs and diseases and from other harmful influences, the potential degree of interference becomes awesome indeed. This ominous giant has been termed "biological engineering". It implies the determination of babies' features and therefore of entire populations: the colour of their eyes, their sex, their physical proportions, their brain capacity. Indeed it has been claimed that, when the restrictive size of the birth-canal is by-passed, the volume of the human brain would rapidly increase: intellectual wonders, physical wonders, geniuses in music and art and science. And all these could be multiplied thousands of times, a thousand persons tailored to a declared specification as Nature now tailors identical twins.

Man is indeed daring fantastically in this new biological revolution. As the biologist tinkers with the processes of fertilization and of early embryonic development, he is tinkering with humanity. Is he not playing God?

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