PERFUSION OF OVARIES IN VITRO AND IN VIVO

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ABSTRACT

This paper discusses in vivo and in vitro ovarian perfusion systems described so far in the literature. The interest is not focussed primarily on the results of these studies but rather on the advantages and disadvantages of the techniques and methods used. Another part of the paper summarizes the points which are most important, in our opinion, to take into consideration when developing an in vitro perfusion technique of the ovary. The last part of the paper gives a description of and some preliminary results from an in vitro perfusion system of the rabbit ovary which is under development in this laboratory.

In a classical paper entitled »Factors concerned in the perfusion of living organs and tissues« Belt et al. (1920) started the discussion of their experiments by stating »We wish to keep constantly in the reader's mind that physiological perfusion of any organ is a matter of extreme difficulty and often great confusion is introduced by such methods which are intended to simplify the study of organ function«. Since this statement is still true to-day, it is appropriate to start this review article by discussing briefly the advantages and disadvantages inherent in perfusion experiments as compared with other types of ovarian studies. It is important to discuss these advantages and disadvantages in relation to the purpose of the experiments. Up to now, the purpose of ovarian perfusion experiments has been to study various aspects of steroid production, steroid metabolism and steroid secretion. The main reason for our group to start ovarian perfusion experiments is, however, the hope that this approach will help us to penetrate further aspects of ovarian metabolism other than
steroidogenesis; *e.g.* ovarian carbohydrate metabolism, ovarian amino acid transport and protein synthesis, and ovarian RNA metabolism. For several years these aspects of ovarian physiology have been studied in our laboratory using various types of ovarian preparations other than ovarian perfusion (*e.g.* Ahrén *et al.* 1969, 1970; Ahrén & Selstam 1971).

In the first part of this article some problems concerning the definitions of the terms *in vivo* perfusion* and *in vitro* perfusion* will also be considered. The second part will summarize the techniques for ovarian perfusion used by various groups from the early experiments by Carrel & Lindbergh (1935) to *personal communications* from groups working with ovarian perfusion today. We will not discuss in detail the results of the experiments but focus rather on the specific aim of the study, the technique used and whether the experimental design has been adequate to the purpose of the study.

The last part of the article will give a description of an *in vitro* perfusion technique of the rabbit ovary which is under development in our laboratory. Some preliminary results with this preparation will be mentioned. As an introduction to this last part of the article, we will summarize the points which in our opinion are most important when working with an *in vitro* perfusion technique of the ovary.

**RELATION TO OTHER OVARIAN EXPERIMENTS. DEFINITIONS**

Our basic knowledge of ovarian physiology derives its origin from classical *in vivo* experiments with the whole animal, *i.e.* surgical extirpation or transplantation of the ovary or the pituitary gland with subsequent analyses of morphological changes in target organs. Another approach to the whole animal experiment has been the analysis of urinary excretion rates and blood concentrations of steroids during various phases of the oestrous cycle, after hypophysectomy, after gonadotrophic stimulation etc. It is, however, difficult to draw any *direct* conclusions from such experiments concerning pathways of ovarian steroidogenesis or how the ovary meets its energy demands under physiological conditions. A useful modification of the whole animal experiment which gives more direct information, at least when working with steroidogenesis, involves cannulation of the ovarian vein with subsequent collection and analysis of the ovarian venous blood. A further experimental modification which can be very useful is the technique of placing a cannula in the ovarian artery so that various substrates (*e.g.* labelled steroid precursors), hormones and other regulatory substances can be injected or infused directly into the
ovary. It is obvious that under these experimental conditions the ovary is still under gonadotrophic, nervous and other endogenous influence.

Collections of ovarian venous blood in connection with cannulation of the ovarian artery have almost exclusively been made in order to analyse steroid biosynthetic pathways and steroid secretion. It is, however, of interest to note that Odeblad and co-workers (Odeblad & Enhörning 1953; Odeblad et al. 1955) used this experimental approach very early in attempts to determine whether ovarian glucose uptake and oxygen consumption could be altered by changes in the degree of gonadotrophic stimulation.

One possibility to minimize endogenous influence in experiments involving collection of ovarian venous blood and cannulation of the ovarian artery is to ligate the ovarian artery above the point of insertion of the arterial cannula, and then perfuse the ovary from a perfusion pump. This technique has been used for the rabbit ovary by Hilliard et al. (see below), and this type of preparation is, from a strictly logical point of view, the only one meriting the classification of an »in vivo ovarian perfusion system«. It is, however, a widely accepted practice to call those experiments combining collection of the ovarian venous effluent with infusion of substances directly into the ovarian artery »in vivo perfusion experiments« although the animal itself provides the ovary with blood from its own intact circulation. There is no need for us to try to change this nomenclature. We suggest, on the contrary, that all experiments involving repeated or continuous collection of the ovarian venous blood be designated as »ovarian in vivo perfusion experiments«, even when the ovarian artery is not cannulated. In this review article, we will call the last mentioned type of experiments »Type I in vivo perfusion«. Experiments involving collection of ovarian venous blood and cannulation of the ovarian artery with circulation intact will be designated as »Type II in vivo perfusion«. A »Type III in vivo perfusion system« will be the one where the artery has been ligated, and the ovary is perfused exclusively by means of some type of perfusion apparatus. In this case the ovary is still in situ and can be influenced by endogenous nervous factors and probably also by humoral factors reaching it via small vascular and/or lymphatic anastomoses which are technically very difficult to ligate.

Ovarian »in vitro perfusion systems« are of course deprived of all endogenous influence. This is in many experiments an advantage. It is, however, important to remember that local factors from the adjacent uterus, at least in certain species, seem to play an important role in the control of ovarian function (e. g. McCracken et al. 1971), and that such factors might, under physiological in vivo conditions, influence and modify the effects of other regulatory factors. The functional significance of the ovarian innervation must also be considered when working with a denervated in vitro system, since in all species investigated the ovary is supplied by adrenergic nerve terminals, and recent
studies have revealed that part of these terminals are of non-vascular nature (Sjöberg 1967, 1968).

There are no general definition problems associated with the ovarian in vitro perfusion systems similar to those discussed above for the in vivo systems. However, a long series of important factors have to be taken into consideration when developing an in vitro system and when interpreting the results from experiments with such a system. These factors are discussed in a subsequent part of this article.

IN VIVO PERFUSIONS

The first type of ovarian in vivo perfusion systems, designated as Type I above, where collection of ovarian vein blood is performed without cannulation of the ovarian artery has been reported in many species. Such experiments will here be mentioned in the four species (dog, sheep, human, and rabbit) where Type II or Type III of ovarian in vivo perfusion has also been developed.

In the dog, Telegdy & Endrőczi (1961) measured progesterone content in ovarian venous blood taken from dogs in various stages of the oestrous cycle, and Telegdy & Huszár (1962) studied the effects of FSH and HCG on the progesterone content of the ovarian venous blood. These investigators did not, however, describe in detail their technique for cannulation of the ovarian vein. Such a detailed description has been given by Romanoff et al. (1962) who measured progesterone secretion by cannulation of the ovarian vein in anaesthetized anoestrus dogs. These investigators collected the ovarian vein blood either continuously throughout the experiment (up to 180 min) or by intermittent sampling of 4–5 hours. Interrupted sampling was possible by cannulation of the ovarian vein below the anastomosis to the uterine vein, since – when the cannula in the ovarian vein was clamped off – the ovarian venous drainage was via the anastomosis to the uterine vein. Romanoff et al. (1962) did not find any effect of acute intravenous injection of HCG on the progesterone production by the cannulated canine ovary, although administration of HCG intramuscularly for 3 days increased the progesterone secretion rate markedly. It is of interest that Romanoff et al. (1962), with their type of preparation, found a significant relation between progesterone secretion and ovarian blood flow rates in most experiments.

In 1963 Depaoli & Eik-Nes developed a Type II in vivo perfusion system for the dog ovary, and a series of experiments using this preparation has been published since then by Eik-Nes and co-workers. Their studies have been
directed to various aspects of the in vivo steroidogenesis by the ovary, and their results have been summarized and discussed in a recent article by Aakvaag & Eik-Nes (1969). In the experiments of Eik-Nes and co-workers all dogs were pretreated with HCG for several days in order to induce oestrus in the anoestrus animal. Attempts were also made to develop the same in vivo ovarian perfusion preparation in the untreated anoestrus dog (Depaoli & Eik-Nes 1963), but they were unable to »obtain an adequate preparation« due to the small caliber of the ovarian veins and arteries in such dogs.

Ovarian vein blood in the sheep was first analysed by Edgar (1953) who measured progesterone chemically in the ovarian venous effluent of pregnant and non-pregnant ewes. A more detailed study of progesterone concentration in the ovarian venous blood of individual ewes at different stages of the oestrus cycle and pregnancy was subsequently published by Edgar & Ronaldson (1958). Short et al. (1963) likewise analysed progesterone secretion from the sheep ovary by cannulation of the ovarian vein. These last mentioned investigators also studied the effect of intravenous injection of gonadotrophins. Recently Mattner & Thorburn (1969) measured ovarian blood flow in the sheep during various stages of the oestrus cycle by cannulating the utero-ovarian vein and ligating all tributaries other than the ovarian vein, so that only ovarian blood entered the catheter. The mean blood flow was 3.40 ± 0.28 ml/g × min during Days 0, 1, 2, 15 and 16 of the cycle, while it was significantly higher (6.62 ± 0.71 ml/g × min) during Days 3 to 14. An interesting finding was that ovaries with and without corpora lutea showed similar cyclic variations in blood flow suggesting that humoral and/or nervous factors determine the blood flow and override the possible local effect of a corpus luteum. It is also important to note the very high rate of ovarian blood flow (300–600 ml/100 g × min) which was reported by Mattner & Thorburn (1969) who used the cannulation technique. This blood flow is several times greater than the capillary flow in ovarian tissue as estimated by indicator fractionation techniques, e.g. Sapirstein's technique (1958), or the krypton dilution technique; by the use of the latter the blood flow of the sheep ovary had been estimated to be 20–40 ml/100 g × min (Setchell 1969) and that of the sow ovary 50–60 ml/100 g × min (Rathmacher & Anderson 1968).

A Type II in vivo perfusion system of the sheep ovary has been developed by Domański and coworkers (Domański & Dobrowolski 1966; Domański et al. 1967), who analysed the effects of FSH, LH and prolactin on the rate of ovarian blood flow and progesterone secretion. Acute perfusion experiments were performed on 2–4 year old ewes on the 8th and 15th day of the oestrus cycle while the animals were under nitrous oxide anaesthesia, and gonadotrophins were administered »into the side branch of the ovarian artery« during a continuous collection of the venous effluent from the ovary. They reported an increased secretion of progesterone after infusion of LH or prolactin, and
a decreased progesterone secretion after FSH administration. In some experiments they found a marked increase in blood flow after LH stimulation (from 100 to 400 ml plasma/h), but this was not a consistent finding.

One obvious disadvantage of the above mentioned in vivo perfusion systems is that the experiments require anaesthesia, and it is quite possible that ovarian metabolism, e.g. ovarian steroid metabolism, might be influenced under these conditions. Another disadvantage is that the useful life of these preparations is short. A very interesting modification of the ovarian in vivo perfusion system is therefore the technique of autotransplanting the ovary of the sheep to a jugulocarotid skin loop in the neck with vascular anastomoses (Goding 1966; Goding et al. 1967; McCracken & Baird 1969). This preparation gives access to both sides of the ovarian circulation in the conscious animal, and it can be used for both acute and long-term investigations. Many experiments have been performed with this interesting preparation in order to study the basal levels of steroidogenesis during the oestrous cycle, the gonadotrophic control of steroidogenesis and steroid secretion rates, as well as luteolytic factor(s). Most of these studies have been summarized recently (McCracken et al. 1971) and will therefore not be mentioned here. The following points, which are of immediate interest for ovarian perfusion studies in general, will be mentioned, however: 1) The values for ovarian blood flow from the transplanted ovary are 20–30% higher than the values obtained in situ by Mattner & Thorburn (see above) during the luteal phase. This difference is likely to result from a blood flow contribution from the loop of skin enclosing the ovary (McCracken et al. 1969) in combination with a probable compensatory hypertrophy of the transplanted ovary following removal of the other ovary (Sundaram & Stob 1967). However, this difference should be borne in mind when the physiological responses of the transplanted ovary are evaluated, since changes in blood flow are in fact significantly underestimated with this preparation assuming a constant contribution from the surrounding skin; 2) altering the temperature of the autotransplanted ovary experimentally produces a decrease in both blood flow and progesterone secretion; 3) infusion of LH directly through the autotransplanted ovary via its arterial supply consistently increases both ovarian blood flow and the secretion rates of progesterone; 4) the effects of LH on ovarian blood flow and steroid secretion rates have different time-relationships, suggesting that the hyperaemia inducing effect of LH is mediated in a different fashion from its steroidogenic effect; 5) no effects have so far been seen after infusion of milligram quantities of cyclic 3',5'-AMP, either on ovarian blood flow or steroid secretion. It is relevant, in this connection, to point out that, as yet, no investigations concerning protein, RNA or carbohydrate metabolism have been reported with this preparation.

Steroid analyses of ovarian venous blood from human ovaries have been

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performed by many investigators (e.g. Seeman & Saracino 1961; Mikhail et al. 1963; Mahesh & Greenblatt 1964; Horton et al. 1966), in cases where laparotomy for breast cancer or gynaecologic diseases has made the collection of ovarian blood feasible. It has not been possible, for technical reasons, to measure the rate of blood flow of the human ovary directly, and experiments with indirect methods, using krypton or xenon, have not been reported in the literature.

Type II in vivo ovarian perfusions with the human ovary have been reported in a few patients (Doouss & Deshpande 1968; Oertel et al. 1968). Labelled steroid precursors have then been injected via the ovarian artery, and venous blood has been collected and analysed.

Collection of ovarian venous blood from the rabbit ovary was reported by Mikhail et al. (1961) who measured progesterone concentration in ovarian vein blood in pregnant rabbits, and by Hilliard et al. (1961) who found progesterone and its metabolite 20α-hydroxy-4-pregn-en-3-one in the ovarian venous blood of oestrus rabbits without functional corpora lutea. Other interesting findings of Hilliard et al. (1961, 1963) are that, after ovulation induced by mating or by the intravenous administration of gonadotrophins, the output of the above-mentioned steroids was immediately elevated in the ovarian vein blood.

In subsequent studies by Hilliard and co-workers (Hilliard et al. 1964, 1967, 1968) a Type III in vivo perfusion system was developed in the rabbit. In this preparation ovarian venous blood was collected while the ovary was continuously perfused with heparinized blood obtained by heart puncture from donor rabbits. The ovary was left in situ but no blood from the rabbit itself was circulated through the ovary during the experiment. Donor blood was infused by means of an infusion pump through a cannula inserted into the ovarian artery at a constant rate of 1.23 ml/min. Hilliard and co-workers have used this perfusion system to clarify, in a very elegant way, the time-relationship of the postcoital release of pituitary gonadotrophins, but they have not discussed the possibility of anoxia or other metabolic changes in the ovary during the period of perfusion. In a personal communication Hilliard (1971) has mentioned that »the perfusion blood must be oxygenated frequently and warmed to body temperature«. Results obtained by recirculation of the blood have not been reported with this preparation.

IN VITRO PERFUSIONS

The first successful attempt to perfuse the ovary in vitro was made as early as 1935 by Carrel & Lindbergh. These investigators have reported in vitro
perfusions of many tissues and organs, but were particularly interested in the endocrine glands (see rev. by Carrel & Lindbergh 1938). The pre-requisite for their studies was a pump, which gave a pulsative circulation of oxygenated fluid. The system was a closed one with re-circulation of the perfusate. They tried various perfusion media, mostly heparinized whole blood and heparinized plasma, but these »natural media« were always diluted to a greater or lesser extent with salt solutions. The purpose of these experiments was to find conditions under which the isolated organ could remain »alive and normal« for a long time, i. e. a period of days. They used two criteria to show that the perfused organ was still »living« after the perfusion: a normal histological picture and the ability of a fragment of the perfused organ to give rise to growth of epithelial cells when cultivated in a flask. By these criteria, they were able to keep the perfused cat ovary alive for several days. They also mention that the isolated ovary could increase in weight, and that corpora lutea could develop.

The above-mentioned time consuming experiments of Carrel & Lindbergh constitute, without doubt, an admirable and important foundation for further studies in this field, even if it is legitimate to question several things, e. g. how normal the ovaries were after several days of perfusion and if the weight increase of the isolated ovary was, at least in part, due to oedema, etc. That the ovary was among those organs which could be perfused for a long time with media lacking erythrocytes is of great immediate interest, however. The adrenal glands and the testes were among the organs which showed more or less degeneration after perfusion in media without red blood corpuscles.

The next series of experiments with in vitro perfusion of the ovary was reported by Werthessen (1949). Although the general technique and the aim of his study were very similar to those of Carrel & Lindbergh, he used erythrocytes in all his perfusion media, claiming that this was necessary to keep the ovary »normal« for a long time in vitro. In addition, Werthessen required »growth and tissue repair« as criteria for viability of the perfused ovary. In experiments with cow and rabbit ovaries, he reported ovulation after addition of »large amounts of gonadotrophins« to the perfusate, but ovulation was not seen in experiments with human ovaries. He did report, however, an increment in the oestrone concentration of the perfusate in some experiments with human ovaries after addition of gonadotrophins. Some years later Werthessen et al. (1953) published the first paper where the in vitro perfusion technique was used for a more detailed study of ovarian steroidogenesis. These authors perfused pig ovaries with 14C-labelled acetate and claim to have found radioactivity in cholesterol, oestrone and 17β-oestradiol. They mention that ovaries were obtained from »one pregnant and one non-pregnant sow«, but they give very few additional details of the experiment.

The next paper dealing with ovarian perfusion in vitro was published in
1962, when Romanoff & Pincus (1962) described a modification of Werthessen's perfusion apparatus which they used for perfusion of bovine ovaries. The ovaries were perfused with homologous whole blood, citrated to prevent coagulation and containing antibiotics to prevent bacterial growth. Their original perfusion apparatus was one with re-circulation of the medium and with a special pulsator which provided a pulsative flow through the organ. The authors claim that pulsating flow is desirable in maintaining blood flow through the ovary for extended periods of time. In further studies of Romanoff and co-workers, summarized in two articles (Romanoff 1966; Bartosik & Romanoff 1969), the technique was, however, modified. One important modification was that the perfusion medium was not re-circulated through the ovaries. With this modification, they wanted to have a more physiological in vitro perfusion technique, in which – as in vivo conditions – the ovarian effluent is not re-perfused through the gland, and the primary products are not substrates for further transformation (Romanoff 1966). This seems to be a very adequate modification when the purpose is to study various pathways of ovarian steroidogenesis or the rate of secretion of various steroids from the isolated ovary, as was the case in the experiments of Romanoff and co-workers. These investigators have reported many interesting results including analyses of the effects of various gonadotrophins (FSH, LH and prolactin) on steroid metabolism and steroid secretion in various types of bovine ovaries. Another modification used by Mills & Morrissette (1970) is to perfuse the isolated bovine ovary with a constant flow instead of a pulsative flow.

Romanoff and co-workers have described in detail how they handle the bovine ovary when it is obtained at the slaughter-house, where the ovarian artery is cannulated as soon as possible and the ovary flushed with chilled citrated glucose saline until the venous outflow is clear. The ovary is then chilled by ice until it is brought to the laboratory and placed in the perfusion apparatus. It is first perfused for a minimum of 2 hours (Bartosik & Romanoff 1969) with blood at 37° C before any samples are analysed or injections of precursors or hormones are made with the hope that a revitalization will occur during this period. Romanoff and co-workers have not mentioned the time elapsing from the moment of the animal's death until they obtain the ovary (i.e. the period of warm ischaemia). Mills & Morrissette (1970) mention that this time period is approximately 40 min in their experiments, a period which must be considered very long and which probably varies at least slightly from animal to animal. It is therefore likely that the condition of the ovary at the start of the perfusion varies considerably between individual ovaries even though the morphological features of the ovaries appear similar. This might explain, at least in part, the marked difference in progesterone secretion rate among individual bovine ovaries by Mills & Morrissette (1970). These investigators found basal progesterone secretion rates of the perfused bovine
ovaries of »early pregnancy« to vary from 0.04 to 14.03 µg/min and those of »late pregnancy« to vary from 0.13 to 19.40 µg/min. Armstrong & Black (1966) have clearly shown that anaerobic preincubation at body temperature of surgically removed bovine ovaries markedly decreased the ability of slices of these ovaries to synthesize progesterone during a subsequent incubation period, whereas »no significant reduction in the ability to synthesize progesterone in vitro resulted from prolonged holding of the tissue near 0°C.«

Nakayama and co-workers (Nakayama et al. 1967) have perfused human ovaries in vitro in an apparatus designed originally for perfusions of the human placenta. The system utilized re-circulation of the medium (whole blood), a perfusion pressure of 100–110 mmHg, and a flow of 10–20 ml/min. Perfusion time was 4 hours. They studied steroidogenesis and found conversion of both [4-14C] progesterone and [4-14C] androstenedione to oestradiol. Histochemical studies of the ovaries after the perfusion revealed that the activity of the enzyme 3β-hydroxysteroid dehydrogenase was maintained, which they considered to indicate viability of the tissue.

The in vivo perfusion experiments of Eik-Nes and co-workers have already been discussed, but this group has in one of their papers described an experiment where ovaries from gonadotrophin injected dogs were perfused in vitro (Engels et al. 1968). In this experiment, the investigators used the same perfusion apparatus which they have used in their experiments with the isolated dog testis. This apparatus was originally described as a closed system with re-circulation of the medium (VanDemark & Ewing 1963), but it has since been modified by Ewing & Eik-Nes (1966) to an open system where no mixing of arterial and venous blood occurs. In their experiments with the in vitro perfused dog ovary, Engels et al. (1968) found a stimulatory effect of clomiphene on the rate of conversion of androstenedione to oestrone and oestradiol. In this particular experiment they used an in vitro perfusion system to prove that the effect of clomiphene, which they had first observed in an in vivo perfusion experiment, was not indirect due to a possible leakage of clomiphene into the general circulation as would be possible in experiments in which ovaries are perfused in vivo.

IMPORTANT FACTORS FOR AN IN VITRO PERFUSION SYSTEM

The object of this section is to summarize the factors which are, in our opinion, important to consider when working with an in vitro perfusion system of the ovary. The relative importance of these factors varies, of course, with the aim
of the study. Our own impression is, however, that these factors have not been considered seriously enough in the in vitro systems described in the literature. It will also be mentioned how we try to let these factors form a basis for the development of an in vitro perfusion system for the rabbit ovary.

Selection of species

It should be possible to define, from an endocrine point of view, the experimental animal, and sufficient amount of ovarian tissue should be available for perfusion and analysis. When working with ovaries obtained at the slaughterhouse a great disadvantage is that the organ has to be chosen from its macroscopic appearance or by estimations of the length of pregnancy. Furthermore, the ovaries are always obtained after a considerable anoxic period.

In the human, the day of the cycle can easily be defined, but normal ovaries from fertile women are of course seldom available.

We have chosen the rabbit as experimental animal for several reasons: Much work has already been done on rabbit ovaries and experimental data can therefore be compared to those obtained in other types of ovarian experiments. In addition, the rabbit is a relatively inexpensive and small animal. Being a reflex ovulator, the rabbit can be controlled as regards ovulation and formation of corpora lutea. The use of rats, a species which from many points of view would be ideal, is at present considered unrealistic because of the delicacy of its anatomical structures.

Prevention of anoxic and mechanical damage

Experimental and clinical work with organ transplantation deals with the conceptions of »warm ischaemia«, the interval between the interruption of circulation and the start of cooling the organ with chilled solutions; and »cool ischaemia«, the period of time when the chilled organ receives no oxygen. Long periods of warm ischaemia can easily produce irreversible changes of the function, while the length of the cool ischaemia is not as critical. This has been thoroughly studied for the kidney. Thus, in dogs warm ischaemic periods of 60–75 min give rise to irreversible damage of the kidney (Porch et al. 1960;
Cohn & Moses 1966), while in work with human cadaveric kidneys a cool ischaemia of up to 7 hours did not affect the time of reappearance of function compared to other kidneys with shorter cool ischaemic periods (Brunius et al. 1968). In experiments with bovine luteal slices Armstrong & Black (1966) found that a long warm ischaemic period seriously affected the progesterone secretion, while no significant reduction in the ability to produce progesterone resulted from prolonged holding of the tissue near 0\degree C.

Our technique of cannulation of the vessels in the rabbit has been developed to reduce the anoxic period of the ovary as much as possible. The period of warm ischaemia is now 1–2 min and that of cool ischaemia less than 30 min. Romanoff & Pincus (1962) pointed out that «rough handling» of the ovary reduces its suitability for perfusion. We have tried to minimize the operative trauma by using small instruments and avoiding blunt dissection.

**Perfusion fluid**

An ideal perfusion fluid should be chemically well defined, meet the energy demands of the ovary, contain buffer systems to maintain optimal pH and possess osmotic properties to prevent oedema. In most of the earlier in vitro perfusions of ovaries, blood has been used as perfusion fluid, sometimes diluted, defibrinated or enriched with nutritive solutions or antibiotics. A solution containing blood has the advantage of a great oxygen binding capacity but may have the disadvantage of aggregation of the corpuscular elements and formation of thrombosis. There are, in addition, always a number of unknown factors in pooled blood, such as hormones, metabolites, precursors and co-factors which make the interpretation of the results more difficult.

We have tried an «artificial» perfusion fluid without erythrocytes to avoid the disadvantages of the use of blood. For chilling and flushing the ovary before it is introduced into the perfusion apparatus, a 5\% solution of low molecular dextran in balanced electrolyte solution containing glucose was used (Perfadex, Pharmacia Ltd.). The perfusion medium has been Krebs-Ringer bicarbonate buffer containing 3 g/100 ml of a low molecular polymer of sucrose and epichlorhydrin (Ficoll, prepared by Pharmacia Ltd., Uppsala) and 5.5 mM glucose. A dextran fraction can probably be used instead of Ficoll when working with the rabbit ovary. We are fully aware that such a «poor» medium might be insufficient for many types of metabolic studies because of its lack of precursors, co-factors etc. Problems with solubility of e. g. steroids have also to be taken into consideration.

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**Flow rate of perfusion medium**

Important questions are whether the perfused ovary receives an adequate amount of perfusion fluid, if the fluid is distributed to all parts of the organ and if the flow rate is constant throughout perfusion.

It might seem rational to mimic the rate of *in vivo* blood flow but the problem is that the ovarian blood flow *in vivo* is a matter of debate. Already the abundant vascular supply of the ovary with many anastomoses between the ovarian and uterine blood vessels suggests a high rate of blood flow. The complex internal vascular structure is subject to great variations during the cycle. Little is known about the significance of the vascular adjustments of the ovary during different phases of the cycle and very few quantitative measurements of ovarian blood flow have been carried out *in vivo*. It was already mentioned above that direct measurements of the ovarian venous effluent blood of the ewe revealed about ten times greater blood flow rates than those found in the same species by indicator fractionation technique and xenon and krypton dilution methods (300–600 ml/100 g × min compared to 20–40 ml/100 g × min). Mattner & Thorburn (1969) suggested the existence of intra-ovarian arteriovenous anastomoses to explain the differences. A considerable source of error in the direct methods of measurement of ovarian blood flow must be the existence of the numerous venous tributaries from the Fallopian tube and from the surrounding fat tissue. These are indeed very difficult or impossible to ligate completely.

We have made a few attempts to measure the *in vivo* blood flow of the rabbit ovary by $^{133}$Xenon clearance technique. The isotope was given intrarterially by cannulating the inferior mesenteric artery and placing the tip of the catheter in the aorta at the origin of the ovarian artery. The results need further analysis but in these preliminary experiments we estimated the ovarian blood flow *in vivo* to be 30–50 ml/100 g × min. Similar measurements have been made in the perfused rabbit ovary *in vitro*, keeping the perfusion pressure at 50–60 mmHg and using rabbit blood of perfusion fluid. On the basis of these experiments, the flow rate through the ovary *in vitro* seems to be of the same magnitude as that *in vivo*. As no definite answer to the question of the true ovarian blood flow can be given, we have chosen a perfusion pressure of 60 mmHg for the preparation (see below), keeping the pressure constant during the whole perfusion period, thus allowing the flow rate to vary. It is then of importance that the perfusion apparatus is designed in such a way that the pressure can be maintained constant throughout the perfusion, and that the flow to the cannulated preparation can be measured at different time intervals during the experiment.
Criteria of viability

The term »viability« is not so easy to define, and it is clear that various investigators have used quite different criteria to classify a perfused organ as »viable«. It has already been mentioned that Carrel & Lindbergh (1938) as well as Werthesen (1949) required »growth and tissue repair« in combination with a normal histological picture as criteria of viability of their perfused ovaries. It is, however, well known that a normal histological appearance does not exclude the possibility of serious changes in the metabolism of the organ. It is also clear that epithelial growth and tissue repair can also be seen in tissues which are quite seriously disturbed in their metabolism.

Continuous cellular supply of oxygen and critical metabolic precursors is a fundamental requirement for normal function. It is therefore desirable to test, in all in vitro perfusion systems, that this requirement is fulfilled. It is, in our opinion, possible to test in various ways that the supply of oxygen is adequate but it is more difficult to find feasible methods to prove that critical metabolic precursors are available to the cells.

Most tissues show increased rate of glycolysis with increased production of lactate when the cellular supply of oxygen is inadequate. Determinations of lactate in the perfusion medium can in such tissues be used as a check of oxygenation. Another possibility is to analyse in the tissue some of the enzymes which are known to react quickly under anoxic conditions. Such an enzyme is glycogen phosphorylase which in all tissues investigated so far exists in two forms, one active only in the presence of 5'-AMP (phosphorylase b), and the other one active also in the absence of this co-factor (phosphorylase a). It is known for many tissues that there is a rapid conversion of phosphorylase b to phosphorylase a under anoxic conditions, and this is true also for the ovary (see below).

Nakayama et al. (1967) estimated with histochemical methods the activity of the enzyme 3β-hydroxysteroid dehydrogenase in the human ovaries which they perfused for 4 hours. They found that the activity of this enzyme »was maintained throughout the perfusion experiment«. This is without doubt a physiological approach to the viability criteria. A maintained activity of this single enzyme can, however, not exclude either a certain degree of anoxia or a lack of metabolic precursors.

Another requirement for normal cellular function is a physiological distribution of water and electrolytes between the intracellular and extracellular compartments. Unphysiological osmotic and/or hydrostatic pressure during the perfusion can easily alter this distribution. Determinations of total tissue water and extracellular space in various phases of the in vitro perfusion experiment is therefore informative.
IN VITRO PERFUSION OF THE RABBIT OVARY. OWN EXPERIMENTS

Methods

Animals. Albino rabbits between 2 and 3 kg were used. Rabbits of other strains have also been operated on but the anatomy varied very little. In our operation technique it is of importance that the two ovarian arteries do not branch from the aorta at exactly the same level. No differences in this respect were found between different strains. Fat rabbits were discarded because a lot of retroperitoneal fat makes the operation more difficult, and it is also essential that a minimal amount of fat is perfused with the ovary.

Operation technique. The rabbits were deprived of food 6–12 hours before operation. The animal was anaesthetized by mebumal given intravenously. When surgical anaesthesia was reached, the abdomen was opened through a midline incision from the symphysis to the xiphoid process. The gut covering the lumbar aorta was cut free and put aside. The aorta was loosened from the bifurcation to 2 cm above the origins of the ovarian arteries, and the lumbar arteries were cut between ligatures. The aorta was then ligated below and above the origins of the ovarian arteries. At this moment the period of warm ischaemia starts.

The aorta was punctured near the proximal ligature, and a heparinized catheter with an outer diameter of 1.7 mm was introduced and secured. Through this catheter chilled Perfadex (see above) was injected. When the ovaries turn pale the period of cool ischaemia begins. During very gentle and slow flushing the ovaries were dissected free. The ramus ovaricus of the uterine artery and 2–3 small arteries to the Fallopian tube were always separated from the ovarian artery by ligatures. A ligature was fastened to the ovarian ligament and the whole preparation, i.e. part of the aorta, the two ovarian arteries and the ovaries, was lifted out and separated into two parts (see Fig. 1).

It is important to point out that the ovaries have to be flushed very gently. Otherwise the capillary bed is in some way damaged. This has been seen by flushing intermit-tently with blood and Perfadex, respectively. The erythrocytes can then be washed out completely only by gentle flushing.

Pre-perfusion. The ovaries were first perfused for about 1 hour at 37°C with oxygenated Perfadex adjusted to pH 7.4. The medium of this so called pre-perfusion was not recirculated. A perfusion pressure of 65 mmHg was used.

Lactate concentration of the effluent perfusion fluid was measured at close intervals during the pre-perfusion period. Fig. 2 shows the lactate production of eight ovaries. It is initially high and varying markedly in all ovaries. After 20–60 min it is in all ovaries less than 3 μg/100 mg × min. The total going flow through the catheter is 1–2.5 ml/min. If the whole medium goes through the ovary, it would mean a perfusion flow of the ovary of 300–1200 ml/100 g × min. It is unfortunately unavoidable to include some fat around the arteries and ovary in the preparation. When flushing intermittently with citrated whole blood and Perfadex it is clearly seen that the erythrocytes are as easily washed out from the fat as from the ovary. Under the microscope it can be seen that many small branches from the ovarian artery to the fat are open. Our inference is therefore that a considerable amount of perfusion fluid does not go through the ovary itself.

After the pre-perfusion the ovary is rapidly moved to the organ chamber of the perfusion apparatus.

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Fig. 7.
Sequences of final part of the operation. 
Left: Aorta cannulated in anterograde direction; Centre: Aorta cannulated in anterograde and retrograde directions and then ligated with double ligatures between the origins of the two ovarian arteries. Right: Aorta divided between the two ligatures. During the whole procedure continuous gentle flushing was carried out using chilled Perfadex. Perfadex was administered from syringes attached to the catheters. The two ovaries were then placed in two separate organ chambers during pre-perfusion and perfusion.

Perfusion apparatus. The perfusion medium which consists of Krebs-Ringer bicarbonate buffer with the addition of 100 mg glucose and 3 g low molecular Ficoll (see above) per 100 ml is circulated in the perfusion apparatus for 15 min before the ovary is placed in the organ chamber. The perfusion apparatus is illustrated schematically in Fig. 8. The temperature of the organ chamber, bubble trap, oxygenator and the gas inlet is 37°C. Adequate pressure is regulated with the pump. The flow from the pump is separated into two parts, one going to the organ chamber and the other one going directly to the oxygenator, the so called »overflow system«. The system is adjusted in such a way that the overflow is 50 times greater than the organ flow. This overflow system permits a very efficient oxygenation and warming of the medium, and it also makes it easy to regulate the perfusion pressure. Samples of the medium are taken from the overflow system. The medium from the organ chamber and the overflow is oxygenated (95% O₂ - 5% CO₂) by the gas counterflow in the oxygenator which ends in a filter. The rate of medium flow through the perfused preparation can easily be measured via the perfusion tube running from the organ chamber.

The ovary is fixed by the ligature in the ligament to the rubber stopper of the organ chamber. The ovary and its cannulated artery are thus suspended in the air-filled organ chamber in such a way that the ovary does not hang below the artery.
Lactate production during pre-perfusion of 8 follicular rabbit ovaries. Perfusion medium was Perfadex (see text), containing 5.5 mM glucose. The medium was oxygenated and warmed to 37°C. It was not re-circulated. Perfusion pressure was 65 mmHg. Flow varied from 1 to 2.5 ml/min.

Special care has been taken not to obstruct the ovarian vein, which is left open. In the apparatus a minimum of 15 ml of medium is necessary to ensure proper circulation. The evaporation from the system is 0.5–1 ml per hour.

Results

In all experiments reported here, 40 ml of Krebs bicarbonate buffer containing glucose and Ficoll (see above) was used as perfusion medium.

Histological examination. Four ovaries were taken for histological examination after 1 and 2 hours of perfusion. No morphological changes from a normal ovary could be seen. No erythrocytes were found in the vessels or in any part of the ovary. The follicles appeared normal and contained oocytes. No oedema was seen.

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Schematic drawing of the perfusion apparatus. 1. oxygenator, 2. filter, 3. pump, 4. bubble trap, 5. manometer connected to bubble trap, 6. organ chamber with the ovary, and 7. »overflow system«. Heating jackets are indicated with oblique lines. Tubes are drawn with solid lines.

**Lactate production.** The glycolysis of isolated prepubertal rat ovaries has been thoroughly investigated in this laboratory (for ref. see Ahrén et al. 1969). Linear production of lactate has been found during 4 hours of incubation in oxygenated medium. Increased rate of glycolysis with increased production of lactate has been found after addition of gonadotrophins and when the ovaries were incubated under anaerobic conditions.

Lactate production during perfusion was determined for 4 rabbit ovaries. The production was linear during 4 hours, the longest perfusion period studied. In these experiments, lactate production was $133.7 \pm 19.2 \mu g/100 \text{ mg} \times \text{hour}$. Two ovaries were first perfused in 95% O₂ - 5% CO₂ for 2 hours and then perfused in 95% N₂ - 5% CO₂ for 1 hour. During the anoxic period, the ovaries more than doubled their production of lactate compared to the oxygenated period.

**Phosphorylase determination.** Phosphorylase has here been determined according to Bueding et al. (1962). Two rabbits were anaesthetized, the abdomen opened, and the left ovary frozen in situ and then analysed. The right ovary was frozen in situ after the artery to this ovary had been ligated for 60 sec. The two ovaries which were frozen without any preceding anoxia showed 25.5 and 28.4 per cent of the enzyme in the a-form, while the corresponding values for the two ovaries analysed after 60 sec of anoxia were 38.7 and 72.0 per cent, respectively. In order to see whether phosphorylase activity is restored to its low in vivo level during perfusion, three ovaries were analysed directly after a two hour perfusion period. It was found that 21.5, 15.5 and 21.2 per cent of the enzyme was in the a-form, thus indicating that the ovary is...
adequately oxygenated during the perfusion period. In the isolated prepubertal rat ovary around 20 per cent is in \(a\)-form under oxygenated conditions. Anoxia results rapidly in a doubling of this form of the enzyme without changes in the total enzyme activity, \(i.e.,\) phosphorylase \(a + b\) (Selstam & Ahrén, to be published).

**Total tissue water and extracellular space.** No macroscopic signs of oedema of the ovaries were seen after the perfusion periods. However, in some cases a slight oedema was clearly seen in the fat included in the preparation. No measurements of total tissue water and extracellular space have been made of the fat.

The dry weight of 8 rabbit ovaries analysed directly without perfusion was 18.7 ± 0.8 per cent of the wet weight. In 13 follicular ovaries perfused 1–2 hours no significant difference was found compared to the \textit{in vivo} measurements, the corresponding value being 21.4 ± 1.0 per cent. The extracellular space of 4 perfused ovaries, determined with \([^{14}\text{C}]\) inulin was 33.8 ± 2.7 per cent.

**Amino acid uptake.** It has been shown that the isolated prepubertal rat ovary has a linear uptake of the non-utilizable amino acid \(a\)-aminoisobutyric acid (AIB), \(i.e.,\) that the intracellular concentration increases in direct proportion to the incubation time (Ahrén et al. 1967). In two rabbits the left ovary was perfused for one and the right for 2 hours with \([^{14}\text{C}]\) AIB added to the medium. The ovaries perfused for 1 hour showed an activity of 1223 × 10^3 and 1727 × 10^3 cpm/ml intracellular water, respectively. The estimations were based on an extracellular space of 33.8 per cent and a dry weight of 21.4 per cent. The two ovaries perfused for 2 hours showed an activity of 3059 × 10^3 and 3478 × 10^3, respectively. This result indicates a linear uptake of this model amino acid in the perfused rabbit ovary.

**Concluding remarks**

With our methods of preparation and perfusion it seems possible to develop an \textit{in vitro} perfusion system of the rabbit ovary, which is suitable for many types of metabolic studies. It is, however, important to point out that we are still in an early phase of this project, although we consider the results of the preliminary experiments mentioned in this section promising.

The perfusion apparatus which is described in this paper can, with only minor modifications, be used for ovaries from other species than the rabbit. Bovine and human ovaries have been tried with success, at least from the technical point of view of the perfusion.

We consider it important to study thoroughly the basic conditions of our \textit{in vitro} perfusion system before initiating experiments with gonadotrophins or other regulators of ovarian function. Such experiments will, however, be started in the future.

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Discussion

Bartosik: In the early perfusions that Dr. Romanoff did, the warm ischaemia time was up to 45 minutes (Romanoff 1966). Some time in 1967, we discovered that we could obtain the ovaries within less than ten minutes after slaughter. In either situation, after the ovarian arteries were cannulated, the organs were flushed with glucose-saline containing anticoagulants and antibiotics and stored in ice-chilled saline until put on the perfusion pump. Furthermore, in either situation, we observed a gradually increasing progesterone secretion rate during the first 60–90 minutes of in vitro perfusion. Accordingly, we routinely perfused the luteal ovaries with fresh whole blood for a minimum of two hours »revitalization time« prior to beginning an experiment (Bartosik & Romanoff 1969a). I must emphasize that the criteria which we have used
for assessing luteal function are the maintenance of both the rate of synthesis and the rate of secretion of progesterone. Nevertheless, we were concerned with the problem of »warm ischaemia« and in order to sidestep the issue, as well as to obtain luteal ovaries of known post-ovulatory age, we attempted in vitro perfusions of ovine luteal ovaries. We obtained the sheep ovaries at surgery and began the in vitro perfusions after a »warm ischaemia« time of about one minute. Under these conditions, we were unable to maintain luteal function for any longer than three or four hours. In terms of a contribution to this symposium, the important point to note is that there are species differences in the requirements for in vitro perfusion of luteal ovaries.

Of considerably more importance than the issue of »warm ischaemia« is the fact that luteal function is dramatically inhibited when the perfusate is recycled through the ovaries (Bartosik & Romanoff 1969b).

Ahrén: I am glad to hear that it is possible to bring down the warm ischaemia also when working with bovine ovaries, but this is apparently not true everywhere, since in the most recently published experiments by Mills & Morrissette (1970), this time period was stated to be »approximately 40 min«. I think that it is important to remember, Dr. Bartosik, that you measured progesterone and nothing else in your experiment. There might still be progesterone secretion if several other aspects of the metabolism have been changed.

Concerning recirculation, I agree that this is a disadvantage from many points of view. However, for our purposes, when we want to study e. g. protein and RNA synthesis, where it is necessary to have labelled and often expensive precursors in the perfusate, I think that this is the type of system we must have. We are aware of the disadvantage and we will take it into consideration when evaluating the results.

Eik-Nes: Do you have any data on the activity of ovarian phosphorylase in animals not exposed to anaesthetics? The reason for this question is that in both the open (Nishizawa & Eik-Nes 1964) and the closed (Engels et al. 1968) ovarian preparation of the dog as developed in our laboratory there appears to be a consistent fall in the rate of both progesterone and Δ4-androstenedione secretion associated with the use of anaesthetics.

Ahrén: We have no such data from our rabbit experiments. In the prepubertal rat we have more extensive studies on the ovarian phosphorylase (Ahrén & Selstam 1971). When the rats are killed by cervical fracture and the ovaries dissected free from bursae and extraneous tissue under a stereomicroscope, a procedure which takes 1-2 min, then the per cent phosphorylase a is 35-50%. When the rats are under pento-barbital anaesthesia and the ovaries are frozen in situ without any anoxic period, then the phosphorylase a is around 20%. Our interpretation is that the higher values after dissection under a microscope are caused by anoxia, and that the low value in the ovaries from the anaesthetized rats is the true in vivo value. I think, however, that also other types of anaesthesia have to be investigated before a definite conclusion can be made.

Urquhart: I am puzzled by your rationale for not doing the dissection of the ovary during the period of time in which blood is flowing. It seems to me that you could do the whole dissection before you start pumping the artificial perfusate and thus have a shorter period of time in which the ovary is exposed to what would seem to be the very uncertain conditions of supplying it with cold perfusate. Then you could, in addition, avoid the problem of having a precipitous drop in perfusion pressure by cannulating the aorta via the iliac artery instead of cannulating from the cardiac end of
the aorta, which necessarily halts ovarian perfusion. Thus, you might avoid some of the period of instability that you have to contend with.

Ahrén: I am not quite sure that I understood your question right, but I think that your question was why we did not cannulate the ovarian artery itself. This is of course possible, but we have the experience that it will then be a very marked spasm in this small vessel. Hilliard and coworkers have cannulated the ovarian artery in their in vivo perfusions of the rabbit ovary, but we think that it can be an advantage to use, at least for an in vitro system, the type of preparation described by us.

Urquhart: I was not advocating that you cannulate the ovarian artery directly. You can avoid even momentary interruption in flow if, instead of cannulating the aorta on the cardiac side of the ovarian artery, you go down and cannulate the iliac and slide the catheter up in a retrograde fashion while the flow still proceeds down the aorta. Then you can establish flow through that cannula and then interrupt the aorta on the cardiac side of the origin of the ovarian artery.

Goding: When we make ovarian transplants, we dissect the ovary free from all other vascular connections and connective tissue attachments before interrupting its arterial supply. In other words, immediately before excising the specimen, its sole attachment is via the ovarian artery to the aorta. All bleeding points in the broad ligament have been controlled, so that the only blood visible at that time is the utero-ovarian venous effluent. As Dr. Urquhart said, you should be able to prepare your ovaries in the same way. I am also bothered about your use of a hand-held syringe for making infusions into the arterial line. How do you limit the perfusion pressure when you do this?

Ahrén: Dr. Goding, we have not measured the pressure when we infuse from the syringe, but we are very careful since we know that a high pressure can destroy the preparation completely. We have considered the possibility to infuse the ovary also during this period from some type of reservoir, but we have not yet tried such a modification.

Coming back to the first question by Dr. Urquhart, why we have not made the whole dissection procedure of the ovaries and the arteries before cutting off the blood supply from the animal itself, the reason is that it might be an abnormal circulation in the ovary as soon as we start to dissect quite close to it due to spasm in the artery itself. It is very important to wash out all blood corpuscles from the ovary with the perfusate, and we have therefore started the infusion of the cold perfusate as early as possible, since we think that the ovary is well preserved as soon as the temperature is brought down by the ice-cold perfusate. I agree that we ought to try the procedure you suggested. It might be better than the one we have tried.

Cedard: Have you measured the phosphorylase $a$ and the glucose level after the addition of chorionic gonadotrophin?

Ahrén: We have not yet made any studies with addition of gonadotrophins or other regulatory substances. We have taken the view that it is better to have the experimental system well worked out and characterized before the start of the more interesting experiments with such regulatory substances. Studies with gonadotrophins will, however, be started in a near future.

Lunenfeld: I have two questions: 1. How long can you continue the perfusion? 2. Since the ovary is a multicompartmental organ and the requirements for the function and
development of the oocyte, the granulosa, theca and stroma cells and the corpora lutea are different, which morphological or functional parameters do you suggest in order to analyze whether damage has occurred to any of these specific compartments?

Ahrén: The ovaries have so far been perfused up to 4 hours, and they seem to perform well during this time. Determinations of total tissue water, extracellular space and phosphorylase activity have, however, up to now been performed just after two hours of perfusion, so we don’t know for sure how good the system is after that time.

Your second question, Dr. Lunenfeld, is a more difficult one, but also a very important one. We have started this project with young virgin rabbits without any corpora lutea in the ovaries. If the project can develop as we hope, then we will also use ovaries in other stages of development, where the rabbit as a reflex ovulator is a good species to work on. We agree that it will be of great importance to investigate whether various compartments of the ovary are more easily damaged than others during an in vitro perfusion. We have so far just taken some ovaries for light microscopy, but we will try in the future to collaborate in this project with an electron microscopist.

McCracken: Last year Dr. Keith Betteridge (1970) published a paper on homotransplantation of the ovary in the rabbit. Part of his procedure was similar to your in vitro perfusion preparation except, of course, he transplanted this to a foreign recipient animal. He was able to stimulate the homotransplanted ovary with exogenous gonadotrophins. I wonder if his technique could be modified in any way to provide a more viable autotransplant for use as an in vivo preparation?

Ahrén: I am glad that you brought up that paper, since it ought to be included in this discussion. Dr. Betteridge dissected, just as you said, the rabbit ovary together with a section of the abdominal aorta and a section of the vena cava, and he transplanted then this preparation into transected analogous vessels of another rabbit. You have with this procedure the same disadvantage of inaccessibility to the ovary as you have with the ovary in situ. It is therefore difficult to see any great demand for such a preparation. Dr. Betteridge transplanted the ovary to hypophsectomized rabbits, and he wanted to explore the sensitivity of this ovary to exogenous HCG. It is, in my opinion, a rather extensive and difficult operation for a somewhat limited question. An interesting point in this paper is, however, that the author tried to transplant the ovary also to the neck of the rabbit in a way similar to that used for autotransplantation of the ovary in the sheep. He had, however, no success with this type of transplantation because of the small vessels.

McCracken: I believe you mentioned in your presentation that one of the disadvantages of the in vivo perfusions system is that the blood is allowed to recirculate. Last year Dr. Rado from our laboratory reported on the use of the autotransplanted ovary of the sheep for the study of oestrogen biosynthesis in vivo. (Rado et al. 1970). We infused labelled androstenedione and testosterone into the ovary via the ovarian artery and we were able to show that LH would temporarily stimulate the conversion of androstenedione (which was the best presursor) to 17β-oestradiol. We obtained the best results in these experiments when the venous blood from the transplanted ovary was not allowed to recirculate, that is to say, we used that ovary as an isolated perfusion system, but the ovary was still perfused with the animal’s own blood. These experiments were of necessity short term ones and it was of course necessary to simultaneously replace the lost blood by giving a blood transfusion from a donor. I just mention this to illustrate that this is another application of the autotransplanted ovary.

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References: