VII WORLD CONGRESS ON FERTILITY AND STERILITY

Tokyo/Kyoto *17-25 October, 1971*

INVITED LECTURES/SYMPOSIA PAPERS

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ABSTRACTS OF
INVITED LECTURES, SYMPOSIA PAPERS
FILMS, EXHIBITS

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INVITED LECTURES

(Abstracts 344—352)

344. Physiology and chemistry of the hypothalamic releasing factors for gonadotropins: a new approach to fertility control

R. GUILLEMIN, *San Diego, Calif., U.S.A.*

It is now well-established that the control of the secretion of all hormones of the anterior lobe of the pituitary gland is to be found in the hypothalamus in the form of several discrete substances of hypothalamic origin known as hypothalamic hypophysiotropic hormones or releasing factors. Of these various hypothalamic hormones, only TRF (or thyrotropin releasing factors) has been fully characterized as the tripeptide amide pyroGlu-His-Pro-NH_2; this molecule has been prepared by total synthesis and is now used in patients as a diagnostic tool in hypothalamo-pituitary-thyroid diseases in which it can be used to stimulate endogenous secretion of TSH unless there is a primary pituitary defect. Secretion of the two gonadotropins LH and FSH, which have been recently characterized as two different though closely related glycoproteins, is controlled either by a single hypothalamic releasing factor having the ability of stimulating the release of both LH and FSH or by two factors (LRF and FRF) which would have closely related structures and which would share in unequal ratios the ability to secrete LH or FSH. Highly purified LRF of ovine origin has been obtained by a multiple step purification sequence, the LH-releasing activity being followed *in vivo* by a radioimmunoassay for rat plasma LH. The material so obtained appears homogeneous within the limitations of the methodology available. It is ninhydrin-negative, Pauly-positive and is easily labelled with ^{125}I. The amino acid composition of two such preparations of ovine LRF following 6 N HCl hydrolysis is as follows: His 1, Arg 1, Ser 1, Glu 1, Pro 1, Gly 2, Leu 1, Tyr 1. The LRF activity is destroyed by incubation with a specific pyrrolidone-carboxylyl-peptidase suggesting that the LRF activity is associated with a peptide with an N-terminal pyroGlu (as in the case of TRF). Highly purified hypothalamic LRF stimulates concomitant secretion of LH and FSH according to two linear functions with apparently different slopes. Secretion of prolactin, the third gonadotropin, the existence of which has recently been established conclusively in the human pituitary, appears to be controlled by two hypothalamic factors PRF for prolactin releasing factor, and PIF for prolactin (release) inhibiting factor; the chemical nature of these two substances is unknown at the moment. Recent physiological data have shown that the hypothalamic releasing factors are exquisitely specific in their mode of action (*e.g.* TRF stimulates exclusively the release of TSH and not of LH or FSH; LRF stimulates the secretion of gonadotropins LH and FSH but not of TSH). When the complete molecular structure of the gonadotropin releasing factors has been established, they will be synthesized with no undue difficulty as they appear to be relatively small polypeptides. They will then become available by synthesis for those clinical cases of infertility in which endogenous secretion of pituitary gonadotropins will be induced by administration of the hypothalamic hormone. Furthermore, it is proposed to synthesize analogues of the gonadotropin releasing factors that should act as antagonists of these gonadotropin releasing factors. Such antagonists, which would inhibit the secretion of LH and FSH, should constitute a new type of contraceptive agents, probably free of unwanted side effects in view of the high affinity of the hypothalamic releasing factors for their pituitary site of action.

345. Early complications of legal abortions in the U.S.

C. TIETZE, *New York, N.Y., U.S.A.*

This paper will present data from an ongoing cooperative study involving about 60 hospi-

tals and extramural clinics in 12 states and 18 cities. The interaction of the following independent factors will be discussed: demographic characteristics of patients; period of gestation; type of abortion procedure; type of institution (hospital/clinic) and care (inpatient/outpatient); type of anesthesia; concurrent tubal sterilization; duration of follow-up; post abortal complications by type, severity, and treatment; emergency admissions of outpatients; and readmissions to hospital after completion of abortion.

346. Health care and family planning

A. KESSLER, *Geneva, Switzerland*

Abstract not received for publication.

347. Steps in the human reproductive process susceptible to regulation and the agents influencing them

E. DICZFALUSY, *Stockholm, Sweden*

In addition to the two most conspicuous processes, spermatogenesis and ovulation, there are many other steps in the human reproductive process which are susceptible to hormonal and/or pharmacological regulation. Among these steps mention should be made of sperm maturation in the epididymis, sperm transport, capacitation and phagocytosis in the female organism, ovum penetration by sperm, tubal and uterine transport and metabolism of the fertilized ovum, and the processes involved in implantation and early embryonic development.

Whereas in animal experiments it is relatively easy to interfere with fertility by a variety of hormonal, pharmacological and immunological means, great caution is needed when attempts are made to extrapolate the results of such experiments to the regulation of human fertility. Many agents which interfere with the reproductive processes in laboratory animals have little, if any, effect in the human.

The available principles for the regulation of human fertility will be discussed according to their most likely mechanisms of action, such as agents interfering with spermatogenesis, ovulation, sperm transport, fertilization, implantation and early embryonic development. The most recent developments in the use of prostaglandins, intrauterine trace elements and new fertility-promoting agents will also be discussed.

348. Gynecological pelviscopy in female sterility

K. SEMM, *Kiel, F.R.G.*

Gynecological pelviscopy was improved essentially by 3 technical innovations: (1) developing of thin optical systems of high capacity; (2) utilization of cold light; and (3) filling up the pneumoperitoneum automatically.

Nowadays a modern gynecological pelviscope has an external diameter of 4 to 6 mm (electric wire included). With that instrument the cosmetically most favorable route through the navel is possible (by perforating the muscle 3 to 4 cm lateral of the navel a hernia in the umbilical region is avoided). The pneumoperitoneum is filled up with carbon dioxide, taken from the CO_2-pneu-automatic. The filling pressure is 8-12 mm Hg with an inflow-rate of 1 l/min.

This scale is indicated by the manometers only if the top of the insufflation-cannula is located in the free abdominal cavity. The manometer indicates immediately if the needletip is not in the free abdominal cavity. Thus the danger of blind puncture is eliminated.

Further precautions like the 'aspiration-test' and the 'sounding-test' exclude the possibility that the trocar is lying in an area of adhesions, or is placed in an intestinal loop. The view through the navel into the pelvis minor with the pelviscope is far superior to the old technique with the laparoscope. In connection with the newly developed intrauterine vacuum-sonde, by which the uterus can be moved in all directions, the genital area can be inspected best. Smaller surgical interventions, for example biopsies from ovarian tissue, section of adhesions, coagulation of endometriosis foci, withdrawing by suction of cysts, and sterilization by coagulation of the oviducts can be performed through a second incision with a trocar 5 mm in diameter.

349. Immunological responses of female animals to sperm

A. GHODA, *Tokyo, Japan*

Numerous reports state that there are antibodies against sperm in serum and/or in vaginal or uterine exudates of certain sterile women. There are also many reports that antibody and infertility can be induced in female experimental animals by inoculation of sperm.

Unfortunately, whether immunity really plays a role in infertility or not, is undecided. One reason is that there is very little evidence that serum antibody or immunoglobulin is excreted in the vaginal and/or uterine tubes and that the exudate contains the IgA class of immunoglobulin which is the only known secretory immunoglobulin.

The purpose of this study is to clarify the correlation of circulating and local antibody especially of the IgA class of immunoglobulin in relation to infertility. It is a well known but often forgotten fact that immunological responses of animals differ according to species, strain and the environmental conditions. Therefore, a comparison was made of the immunological responses of mice and rabbits of various strains and in different environmental conditions.

Rabbits and mice in conventional, gnotobiotic or germ-free states were immunized with sperm of homologous or heterologous strains with varying doses and schedules and subsequently the change in the pattern of serum globulins and sperm-agglutinating and immobilizing antibodies in serum and tissue extracts were examined. Mating experiments using sperm-immunized female mice were also performed.

When inbred rabbits of SPF strain were immunized intravenously with ICR strain of conventional mouse sperm, a marked decrease in alpha- and betaglobulin was observed, but gammaglobulin neither decreased nor increased. When rabbits of the same strain were immunized with seminal material, a marked increase in gammaglobulin was observed. The immunoelectrophoretic pattern of sera also showed a decrease in the number of lines following immunization with sperm and it returned to normal 14 days after the last injection.

When immobilizing and agglutinating antibody was examined in the ICR strain of conventional mice, the titer increased with age without any treatment, but when the same strain of mice was inoculated with homologous sperm of very low density, the titer was lower than that of the non-immunized groups. The mating experiments using those mice showed no effect on immunization.

When germ-free mice of the ICR strain were immunized with sperm and mated, the rate of conception was lower than that of the non-immunized groups.

The correlation of serum and/or tissue extract antibodies with infertility will be discussed in detail.

350. New developments on the mechanism of gamete transport in the mammalian oviduct

R. J. BLANDAU, *Seattle, Wash., U.S.A.*

In some mammals (rat, mouse, hamster) the egg and its cumulus mass is shed free from the follicle at ovulation. In the rabbit, cat and monkey the cumulus oophorus containing the egg adheres to the stigma and remains attached to it unless it is removed by the action of the cilia lining the fimbria. The various ways in which the egg is ovulated and transported into the osteum of the oviduct will be shown cinematographically. The eggs of various mammals are transported through the osteum of the oviduct by ciliary action. From there they are transported to the isthmo-ampullary junction by segmental peristaltic contractions of the oviductal musculature. The average time required for eggs to be transported through the ampulla is 6 ± 0.3 minutes. The endocrine mechanism which programs normal egg transport is related to estrogen deprivation and the action of progesterone acting in sequence. Observations on normal and experimental egg transport will be shown cinematographically.

351. Prostaglandins and human reproduction

S. BERGSTRÖM, *Stockholm, Sweden*

The prostaglandins constitute a family of compounds that are biosynthesized in most mammalian cells. They seem normally to exert their main regulatory functions locally in the tissues where they are formed. They are very rapidly inactivated when present in the circulation.

A short review of their metabolism and their many physiological activities will be presented.

The basis for their use as drugs to influence processes related to human reproduction and implications for future developments will be discussed.

352. Fertilization and development of preovulatory human oocytes *in vitro*

R. G. EDWARDS, *Cambridge, United Kingdom*

Early work showed that human oocytes could be matured and fertilised *in vitro*. It is known that animal oocytes matured *in vitro* fail to give rise to normal fetuses, and it is necessary to recover preovulatory oocytes from the ovary if development to full term is needed. We have now controlled oocyte maturation *in vivo*, and remove the oocyte by laparoscopy 2-3 hr before ovulation. Fertilisation of these oocytes has led to excellent cleavage of embryos, especially in Ham's F_{10} with fetal calf serum. Morulae and blastocysts have been found, although a great deal remains to be found out about the culture media, the chromosome complement of the embryos and other factors involved in normal development of the embryo.

352a Physiology and Physiopathology of the Fallopian Tube

C. THIBAULT, *France*

Abstract not received for publication

SYMPOSIA

Chapter 1

Endocrine control of reproduction

Chairman: J. FERIN, Belgium / *Moderator:* L. MARTINI, Italy

(Abstracts 353—363)

353. The action of hormone metabolites: a new concept in endocrinology (on the metabolism and the activity of testosterone)

E. E. BAULIEU, *Bicêtre, France*

The most recent findings suggest that testosterone is metabolized locally in the prostate cells, and that a receptor exists for at least one of the metabolites (androstanolone). Since the various derivatives have different biological properties, the following hypothesis can be proposed:

Testosterone *per se* is not the active hormone (*cf.* the derivation of this word from ὠρμειν, to excite). In the target cells, it is transformed into derivatives (androstanolone and 3β-androstanediol) whose effects are different, but the summation of their action reflects that of testosterone. The distinction permitted by these ultimate transformations of the hormone, perhaps modulated by the cells, could possibly prove to be a very subtle regulation mechanism, an eventual source of pathological disorders and, finally, a possible target for an attack by a therapeutic agent.

The general significance of this concept is discussed as far as other hormone and vitamin D metabolites are concerned.

354. Ovarian response to gonadotropin-stimulation followed by plasma steroids

G. BETTENDORF, F. LEHMANN and A. NEALE, *Hamburg-Eppendorf, F.R.G.*

HMG treatment is performed in patients with hypogonadotropic amenorrhea, and in patients with amenorrhea or anovulatory cycles but with gonadotropin excretion values in the normal range, without response to clomiphene. Treatment was individualized. Parameters for the evaluation of the ovarian response: (1) improvement of cervical factors and (2) the daily estrogen excretion. HCG is administered when maximal ferning is achieved and/or estrogen values exceeding 70 μg/24 hr.

Furthermore plasma estrogens determined by RIA (solid phase method), progesterone and 17-hydroxyprogesterone by a protein binding method, were used for better control of ovarian response. The plasma estrogen pattern during the treatment was quite similar to the urinary excretion pattern. A great variation of time and amount of plasma progesterone increase after induction of ovulation was found. As in spontaneous ovarian cycles, a preovulatory peak of 17-hydroxyprogesterone occurred. This might be a criterion of full follicular maturation and will allow a better timing of HCG administration. From the results obtained so far the authors will discuss different types of ovarian reaction on gonadotropin stimulation.

355. Time relationship between ovulation and hormone levels in plasma

I. DYRENFURTH[1], K. THOMAS[2], M. WARREN[1], R. VAN DE WIELE[1] and J. FERIN[2], [1]*New York, N.Y., U.S.A. and* [2]*Louvain, Belgium*

An attempt has been made to establish a more precise time-relationship between plasma levels of sex hormones and follicular rupture.

In cyclic women, treated with HMG-HCG, or not treated, plasma samples were taken every 2 to 6 hr during the expected ovulatory period. Determinations of the following hormones in plasma have been carried out: LH, FSH, 17β-estradiol and estrone by radioimmunological techniques, and progesterone by competitive binding procedure.

Simultaneously, the patients were submitted to laparoscopy or laparotomy. The age of the

ripe follicles or of the newly formed corpus luteum was appraised by histological or histoenzymological criteria. The protein content of the follicular fluid was also assessed.

356. Factors influencing luteal function

R. O. GREEP, H. R. BEHRMAN, N. R. MOUDGAL, G. J. MACDONALD and K. YOSHINAGA, *Boston, Mass., U.S.A.*

Luteal function may be influenced by several inputs including pituitary gonadotropins, ovarian steroids, prostaglandins, uterine luteolysin, antisera to luteotropins and various chemical inhibitors of steroidogenesis. The authors have been concerned with the mechanisms whereby these substances stimulate or otherwise alter the luteal biosynthesis of progestins and the resulting manifestations of luteal function. Among the acquisitions which luteinization entails are enzymes for the synthesis and hydrolysis of cholesterol esters to yield precursor for steroidogenesis, and another, 20α-hydroxysteroid dehydrogenase, for the conversion of progesterone to its inactive form 20α-dihydroprogesterone. The authors have studied the effect of most of the above agents on these parameters, and have gained new insight as to how these luteal regulators act, and new possibilities for the control of luteal function as a means of limiting fertility.

357. Chemistry and mechanism of action of gonadotropin-releasing factors

M. JUTISZ, *Paris, France*

Two hypothalamic principles are known for their ability to regulate the gonadotropic function of the anterior pituitary gland, LH-releasing factor (LRF) and FSH-releasing factor (FRF). The most widely used method for purifying releasing factors (RFs) consists of the following steps: extraction of stalk and median eminence with 2N acetic acid, gel filtration on Sephadex G-25, desalting by phenol extraction and chromatography on CMC. Schally's group have recently added three more steps to this procedure: free flow electrophoresis, countercurrent distribution and partition chromatography. Highly purified preparations of LRF and FRF from different species have been obtained, but to our knowledge, nobody has obtained preparations sufficiently homogeneous for structural studies.

It seems now well established that LRF and FRF are small, slightly basic peptides, with molecular weights of 600 to 2000. They are inactivated by some proteolytic enzymes but are thermostable. LRF is not destroyed by thioglycolate (no cystine present); its N-terminal amino acid is presumably pyroglutamic acid. Recently, Schally *et al.* reported that porcine LRF (LH-RH in their nomenclature) obtained in a high state of purity, stimulates the release of both LH and FSH. They indicated that the histidine or tyrosine residue (or both), but not tryptophan, is necessary for biological activity of LH-RH and that its carboxyl terminus may be blocked, carboxypeptidase A and B being without effect.

LRF stimulates the release of LH *in vivo* within a few minutes following i.v. injection. The maximum is observed at 10-12 min. Porcine LRF also induces a release of LH in man (no species specificity, Kastin *et al.*). Most of the author's results on the mechanism of action of LRF and FRF obtained by *in vitro* methods can be summarized as follows: RFs exert their primary effects on the release of gonadotropins stored in the pituitary cells; there is good evidence that cyclic AMP participates in the mechanism of action of both RFs; the effect of an RF is dependent upon the presence of Ca^{++}; the activities of RFs are not abolished by inhibitors of RNA and protein biosynthesis. There is no evidence that the synthesis of pituitary gonadotropins is under the direct control of the RFs. Incorporation experiments show that synthesis of FSH and LH can be enhanced by FRF and LRF as well as by a nonspecific factor such as high K^+ concentration in the medium.

358. Studies on hypothalamic estradiol receptor

J. KATO, *Tokyo, Japan*

Isolation of estradiol-binding receptor from hypothalamic cytoplasm of adult female rats by means of sucrose density-gradient sedimentation implies the validity of the hypothesis of the presence of the estradiol receptor propqsed on the basis of the preferential uptake of the steroid by the tissue *in vivo* (Eisenfeld and Axelrod, 1966; Kato and Villee, 1967) and *in vitro* (Kato, 1970).

Sedimentation coefficients of the estradiol receptor in the hypothalamus were 8.6 ± 0.13 (mean and s.e., $N = 9$). The decreasing order of steroid specificity was as follows: estradiol,

diethylstilbesterol ≫ estriol and estrone. The binding of labelled estradiol to the receptor was not affected by progesterone (10^{-5}M), but was decreased in a concentration of 10^{-4}M. Parallel relationship seems to be found between estrogenicity of the hormones and their binding to the estradiol receptor. Macromolecular interaction was completely abolished by pronase, and was decreased to a certain degree by ribonuclease. No effect of deoxyribonuclease was observed. The nature of hypothalamic estradiol receptor is partially protein, at least.

Intracerebral localization of the estradiol receptor was further studied. The specific radioactivity of the receptor was in the following decreasing order: the preoptico-anterior hypothalamus, the anterior part of the hypothalamus, the whole hypothalamus > the median eminence ≫ the posterior part of the hypothalamus, the middle hypothalamus ≫ the posterior hypothalamus. There was no receptor in the cerebral cortex. Sedimentation coefficients of the estradiol receptor in various parts of the hypothalamus were almost the same; the preoptico-anterior hypothalamus, 8.4 ± 0.07 (3), the anterior part of the hypothalamus, 8.7 (2), the median eminence, 8.4 ± 0.38 (4) and the anterior hypophysis, 8.3 ± 0.32 (5). These results demonstrate that the estradiol receptors are predominantly localized in the preoptico-anterior hypothalamus of the rat. The preoptico-anterior hypothalamus being critical for the normal cycle in rats, and being regarded as the principal site for the negative feedback by estrogen, interaction of estrogen with the estradiol receptor in the preoptico-anterior hypothalamus may play an important role in an initial step of the mechanism of feedback action of estrogen on the hypothalamus and in the regulation of cyclic changes of the hypothalamo-hypophyseal system.

(Supported by a grant from the Population Council, M 67. 121, M 71. 34C).

359. Induction of ovulation with human gonadotropins: analysis of results

B. LUNENFELD, V. INSLER and M. SNYDER[1], *Tel-Hashomer and* [1]*Ramat-Gan, Israel*

The results of attempts to induce ovulation by human gonadotropins in 2135 patients over 5006 treatment cycles will be analysed. 792 pregnancies resulted from this treatment. When these data were analysed with regard to the efficiency of treatment and pregnancy rate, significant differences were revealed among the results reported by various investigators. The efficiency, as expressed by the mean number of treatment courses required per pregnancy, ranged from 4.1 to 14.1. The pregnancy rate ranged between 24 and 75%. Selection of patients, treatment schedules and methods of monitoring used by different groups, which might explain some of the differences, are discussed. Comparison of data from various centers revealed that in contrast to the pronounced differences in the efficiency of treatment, the differences in complications of gonadotropic therapy were negligible. The incidence of hyperstimulation was reported to be between 1.3 and 2.5%, the multiple pregnancy rate 28 and 34% and the abortion rate was about 25%.

On the basis of the accumulated results, guidelines for selection of patients and treatment schedules to be used will be suggested.

(This study was supported by Ford Foundation Grant No. 67-470.)

360. Control of implantation

G. MAYER, *Bordeaux, France*

Abstract not received for publication.

361. Regulation of the secretion of gonadotropins and prolactin by hypothalamic neurohormones

S. M. MCCANN, C. P. FAWCETT, A. O. DONOSO, P. S. KALRA, S. KALRA, M. QUIJADA, H. P. G. SCHNEIDER, J. T. WATSON and L. KRULICH, *Dallas, Tex., U.S.A.*

It is now established that a family of neurohormones localized in the hypothalamus regulates the output of anterior lobe hormones. Highly purified LH-releasing factor (LRF) prepared in the authors' laboratory is active *in vitro* in releasing LH at less than 2 ng peptide per gland as determined by radioimmunoassay (RIA). The most potent preparation also has FSH-releasing activity. LRF appears to be a small peptide. FSH-releasing factor (FRF) and LRF are localized to a medial basal zone of the hypothalamus and preoptic area extending from the suprachiasmatic region to the pituitary stalk with the greatest activity in the median eminence (ME) (*in vitro* assay with RIA). Prolactin-inhibiting factor (PIF) is localized to the lateral preoptic area and prolactin-RF(PRF) to the ME.

Utilizing electrochemical stimulation in Nembutal-blocked proestrous rats, it is possible to evoke LH release by stimulating medially in a region extending from the preoptic area to the ME, whereas FSH release is evoked from a region localized slightly more caudally in the anterior hypothalamic area and basal tuberal region. Thus, LH release can result from stimulations that have no effect on FSH release. Electrolytic or radiofrequency ME lesions cause an immediate and sustained increase in prolactin release. A transient release of FSH and LH also occurs followed by a permanent reduction in plasma levels which is more pronounced for LH than for FSH. Suprachiasmatic lesions cause a less marked depression of FSH and LH levels. In such animals the negative feedback of gonadal steroids is intact whereas the stimulatory effect of progesterone on gonadotropin release is blocked. The pituitary responds to RF's with an immediate release of hormone indicating a primary action on release. Thus, in male rats plasma LH rises and prolactin falls almost immediately following injection of crude hypothalamic extract. Release of RF's from the hypothalamus appears to be under adrenergic control.

Dopamine (DA) augments release of FRF, LRF and PIF by hypothalami incubated *in vitro*. Injection of DA into the 3rd ventricle increases plasma LH and decreases plasma prolactin. Increased levels of circulating LRF are found in plasma of hypophysectomized rats following the intraventricular injection of this catecholamine. Estrogen can block the release of LRF evoked by DA both *in vitro* and *in vivo*. Studies with drugs that alter catecholamine biosynthesis indicate that DA is a transmitter to decrease prolactin release. On the other hand, similar studies indicate that the stimulatory effects of progesterone on FSH and LH discharge may be mediated via norepinephrine (NE) acting as a stimulatory transmitter to release FRF and LRF.

362. Theses and hypotheses of ovulation and its preparation: the gonadotropin-estrogen balance

K.-D. PAESCHKE, *Göttingen, F.R.G.*

The author believes that follicle maturation is an acyclic, autonomic process. The cyclic development of the follicles and their regression ('Follikelreduzierung') are controlled by gonadotropic mechanism. In his experiment the author applied high doses of estrogen. Instead of the expected inhibition, an increased rate of ovulation was observed. Therefore FSH cannot be made responsible for follicular development, but it is expected that a potential equilibrium between the two exists. Graafian follicles are producing an excess of estrogen depressing the blood level of FSH. It is expected that there is an increase of hormonal production in the ovaries and secretion of gonadotropins at the same time. The author follows the hypothesis that it is the LH which induces ovulation quantitatively, the rate of which may be specific for each species. The statements made on results of application of estrogens seem to indicate a very complex and partly contradictory process. Between estrogens and FSH there is a functional equilibrium, between estrogens and LH a rebound-phenomenon. An estrogen ovulation will be discussed.

363. 'Feed-back mechanisms' and hypothalamic function

V. D. RAMIREZ

Abstract not received for publication.

Chapter 2

Immunological factors in reproduction

Chairman: E. G. EDWARDS, U.K. / *Moderator:* J. S. BEHRMAN, U.S.A.

(Abstracts 364—370)

364. Formation of immune bodies in the female during the reproductive process

K. BRATANOV, *Plovdiv, Bulgaria*

Immune bodies influencing the reproductive process in males and females have been studied.

The role of spermatozoa, seminal plasma, whole semen, testicular tissue, ova, follicular fluid, ovarian tissue, zygote, embryo, fetal fluids and membranes, sex hormones, blood serum, egg yolk, etc., containing high molecular proteins and inducing the formation of auto-, iso- and hetero-antibodies has been investigated. It was found that immune bodies may be natural or acquired, temporary or permanent. In the normal reproductive process they were within the limit of the immunological tolerance.

Semen, representing a complex antigenic mosaic, and its components — spermatozoa and seminal plasma — containing different antigens (blood group, individual, organ) have also been studied. These antigens under certain conditions induced the formation of auto-spermoantibodies which were the cause of several forms of male sterility. Natural isospermo-antibodies in the female showed usually a low titre (1:16), but in the case of high titres (over 1:512) permanent sterility was observed.

It was also established that zygote, embryo and fetus, embryonic and fetal fluids possess specific antigens which might immunize the pregnant organism. In some cases the immunological tolerance was disturbed and pregnancy was interrupted. The study of immune bodies in the female can elucidate some unexplained disorders of the reproductive process.

365. Transmission of immunity from mother to young

J. E. BUTLER, *Iowa City, Ia., U.S.A.*

The embryos and fetuses of all higher vertebrates reside in an environment which shelters the organism from most of the antigenic stimuli confronting the mother. Probably for this reason the humoral particularly, but also the cellular immune response, is slow to develop and the newborn organism enters the world with little capacity to respond to antigenic stimuli. Death resulting from rampant infection would be the fate of the newborn organism were it not for immunity attained passively from the mother. Passive humoral immunity can be transferred before birth *in utero* in some mammals or from the yolk in birds. Transmission after birth in mammals is by way of the milk.

Mammals can be grouped into at least 3 categories on the basis of their mode of transmission of immunoglobulins. Humans, monkeys, rabbits and guinea pigs acquire nearly all of their maternal immunoglobulins *in utero* entering the world with a serum level equal to that of the mother. Ruminants, on the other hand, derive no maternal immunoglobulins *in utero* and depend entirely on the absorption of large amounts of colostrum during the first few hours after birth. Rats and mice, and probably cats and dogs, acquire maternal immunoglobulins both prenatally and postnatally. The mechanism by which *in utero* transmission occurs varies. In rabbits, rats and guinea pigs transmission is by way of the uterine lumen and fetal yolk-sac while in man transmission is mainly by way of the allantochorionic placenta. Among those organisms that acquire immunity via the milk, variations in the composition of milk and the selectivity and duration of absorption from the gut occur. While absorption by the rat gut is selective and continues 18 days postpartum, absorption by the calf is not selective and is terminated before 48 hr. In ruminants that acquire their immunity exclusively from milk, 7S IgG1 is the principal immunoglobulin in milk while IgA is the predominant lacteal immunoglobulin in all other species. 7S IgG is also the principal immunoglobulin transmitted to the fetus *in utero* in those species using this method.

In all species, except the gut of the newborn calf, there is selectivity in protein transmission. γ-Globulins are transmitted more readily than α-globulins and albumin, and homologous proteins are usually selected over heterologous proteins. The rat and mouse select rabbit and bovine IgG2 over IgG1 while the bovine mammary gland selectively accumulates IgG1. Selection appears to depend on receptor sites which recognize sequences on the Fc portion of IgG immunoglobulins.

While the transmission of immunoglobulins to the fetus *in utero* and the absorption of immunoglobulins from the gut are time dependent, little is known of the regulation of these phenomena. The half-life of absorbed immunoglobulins is longer in neonates than in adults and catabolism increases to approach adult rates near the termination of absorption. The disappearance of enzyme inhibitors and/or permeability enhancers from maternal lacteal secretions or the development of new enzyme systems by the offspring may regulate absorption. Selective accumulation of IgG1 in bovine colostrum appears to be regulated by estrogen and progesterone levels.

The immunosuppressive role of passively administered antibody, the interference of some γ-globulin in the transmission of other γ-globulin, the role of the IgA-mediated secretory immune system in maintaining mucous membrane integrity and the allotypic suppression or

enhancement of the immune response of the young animal are topics that require additional study. The value of maternal antibodies of human milk in maintaining a healthy intestinal environment in the child and the problem of food hypersensitivity in infants are both related to the issue of passive immunity. Finally, a lucid understanding is needed of the exact mechanisms involved in the various processes described so that sound medical treatment can be administered.

366. Antibodies affecting fertility in males

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The presence and titre of sperm agglutinins in the blood of 400 men in sterile marriages, 500 men in fertile marriages and 500 men unselected with regard to fertility have been investigated and the results given in earlier reports. An accumulation of men with sperm antibodies in their blood, particularly at high levels, was found in the sterile group, in comparison to the fertile group and the group unselected with regard to fertility. Statistically significant interrelations were found between the level of sperm antibody in the blood, the degree of sperm motility and sperm agglutination in the ejaculate, the cervical mucus penetrating ability of spermatozoa, and sterility.

Another series of 800 men in sterile marriages was investigated for sperm antibodies with a modified Kibrick method. Semen from another donor was used for this investigation, and the results were read by another person. The incidence of sperm agglutinin positive men was higher (11% compared to 7% in the earlier study). The incidence of men with high titres ($\geq$ 1:64) was 4%, *i.e.* the same as in the earlier investigation. The spermatozoa of the men with high titres showed a reduced cervical mucus penetrating ability.

The finding of a high sperm antibody level in the blood of a man indicates the presence of a serious infertility factor. Because this factor is rather common in the sterility clientele, routine examination for sperm antibodies is recommended. The method of Kibrick is a reliable method for this investigation.

367. The nature of antibodies against spermatozoa found in women of unexplained sterility

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1. Sperm agglutinins found by either Franklin-Dukes' micro-agglutination or Kibrick's agglutination tests, were present even in sera of fertile women as they were in sera of women with unexplained sterility. Sperm immobilisins, however, were found only in sera of 'unexplained' sterile women and showed very similar natures as antibody. It remained in the Visking tube after dialysis, fractionated in γ-globulin, absorbed with washed human spermatozoa and eluted from these spermatozoa by heating at 60°C for 20 min.

2. From our study it was shown that the human seminal plasma contained 3 seminal-plasma specific antigens and 1 or 2 (probably 2) antigens shared with human milk proteins, besides serum proteins and other organ common antigens.

3. The sperm immobilisins were supposed possibly to be the autoantibodies against milk antigens contained in seminal plasma, but it was proved that they were against seminal plasma specific antigens or spermatozoa, and not against milk antigens.

4. Most patients who had the sperm immobilisins in sera showed abnormal Huhner (post-coital) test, and IgG, IgM and IgA were found in their cervical mucus. So far, it is not proved conclusively whether or not these immunoglobulins could be the antibodies against seminal plasma or spermatozoa, even though the transmission of homologous rat anti-sperm antibody from serum to uterus was shown directly by means of paired label techniques in our animal experiments.

368. Antibody titers in male and female experimental animals immunized with sperm antigens

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100 male guinea pigs were injected with ASA (aspermatogenic antigen) in Freund's adjuvant or adjuvant alone. Sera were collected at weekly intervals at exsanguination or by ear bleedings up to 8 weeks. The sera were titered by a modification of Ingraham's PHA (passive hemagglutination test) and correlated with the degree of seminiferous epithelial depletion. In the ASA-injected animals, the titers averaged as follows, $\times 10^3$ at each week: 10; 6; 50; 12,000; 18,000; 30; 3000. These results will be discussed from the point of view of the aspermatogenic syndrome and implications regarding fertility and sterility as well as population control.