Proteins and Steroids in Early Pregnancy

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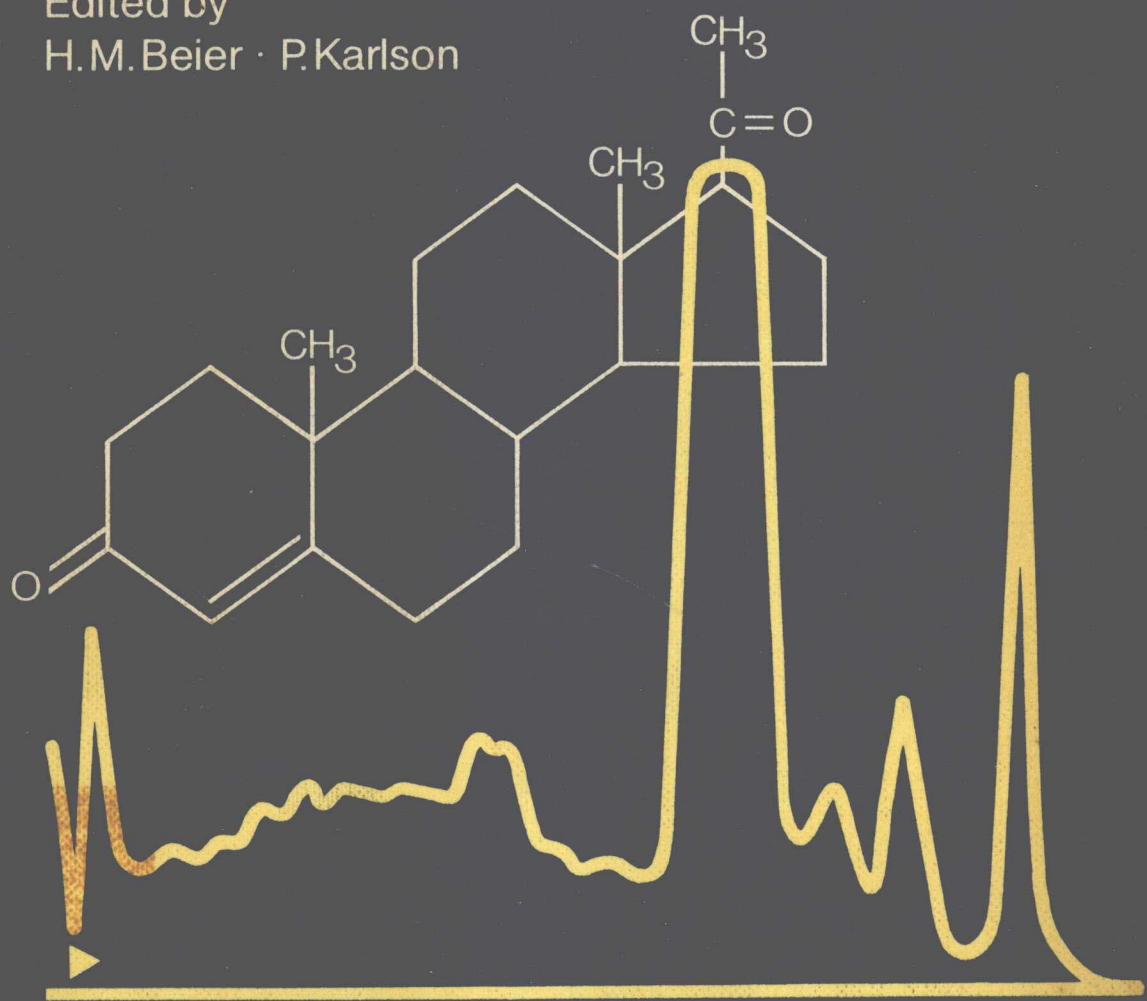

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Preface

It is about 15 years since the first presentation on *uteroglobin* was given to a group of developmental biologists, reproductive physiologists, and geneticists who had gathered in November 1966 at Konstanz (Germany). In the following decade so much knowledge was accumulated that a special symposium seemed appropriate. This was organized as a satellite symposium to the International Congress of Endocrinology at Hamburg and brought together 50 scientists at Aachen. These scientists, working in the field of proteins and steroids in early pregnancy, recognized the impact of what had been reported, and many of them later agreed to contribute to this book and thus to present their research data available until December 1980.

The present volume covers a relatively broad spectrum of data and observations which shed some light on *preimplantational embryonic life* and on the supports and obstacles provided by the maternal organism with respect to final accomplishment of normal implantation and establishment of pregnancy. The book will serve both as a *textbook* and as a *scientific dictionary* for Ph.D. students, postdoctoral fellows and advanced scientists working in this area.

The course of *early pregnancy* depends very much on a proper balance of steroid hormones, and the induction of *protein synthesis* by *steroid hormones* is one of the well-known fundamental processes in cellular differentiation and embryonic development. This process involves the machinery of transcription and its control by steroid hormone receptor complexes, details of which are presented and discussed in this volume. We hope that the present compilation will stimulate designs for new experiments aimed at providing insight into the molecular biology of protein-steroid interactions as well as the control of gene expression.

It is not unlikely that from a practical point of view there will be an explosion of interest in steroid-controlled proteins which support early pregnancy and enable the mother-to-be to provide an *appropriate pregnancy milieu* for the conceptus. Clinicians will desire more detailed knowledge about the significance of steroids and proteins during the establishment of pregnancy. The conditions for egg transfer and blastocyst development will be elucidated to the benefit of those patients who can only have an own child if fertilization is accomplished extracorporally and if the conceptus is re-transferred to its mother within a few days. The data on our basic research on proteins and steroids controlling uterine physiology and embryonic development presented in this book could serve as a really solid basis for further clinical work.

Any success this publication achieves will be due to the contributors who wrote the manuscripts, to the patience of our families, who sacrificed so many weeks and months for the editing work, and finally to the Springer Verlag, who took over the publication of this book.

Aachen and Marburg/Lahn
May 1981

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Contents

Proteins and Steroids in Early Pregnancy: General Considerations

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There are many relations between steroids, more specifically steroid hormones, and proteins. To evaluate the relevance of these interactions for early pregnancy, it may be useful to list at first these most important phenomena:

1. Adenotropic Hormones. The production of steroid hormones is under the control of the pituitary. The pituitary hormones are either peptides (e.g. corticotropin) or proteins. Of relevance in our context are the gonadotropins, glycoproteins consisting of two subunits with different functions – one binding to the membrane receptor, the other entering the cell and being responsible for the physiological action. Pituitary control of steroid hormone synthesis and release is important for the cooperation of various hormones in the sexual cycle, resulting in ovulation, as well as for the development of early pregnancy.

2. Enzymes of Biosynthesis. Steroid hormones are the end-products of a biosynthetic pathway involving many enzymes. Cholesterol may be regarded as the common starting material, since cholesterol is present abundantly in biological membranes of all cells (it is, of course, produced from acetyl-CoA in a long sequence of reactions). For the conversion of cholesterol to progesterone, four enzymes are needed; even more are necessary for the biosynthesis of estrone. It must be kept in mind that steroid hormones are not stored in the hormone-producing tissue; thus the control of biosynthesis is the way of controlling hormone production.

3. Transport of Steroid Hormones. Many steroid hormones are only sparingly soluble in water. In blood as well as in other body fluids, they are kept in solution by special binding proteins. This is not only of importance in the blood, where the steroid-binding proteins have been extensively studied, but also in other fluids including the uterine fluid. Is binding and transport of progesterone one of the main functions of uteroglobin? Perhaps the reader will find a tentative answer to this question in this volume.

4. Metabolic "Activation" in the Target Tissue. Some of the steroid hormones are converted in the target tissues to molecules that exert the physiological action. Specific enzymes (proteins) are involved in this "activation". A well-known example is testosterone: It is the main circulating androgen, but in many target tissues (prostate, seminal vesicles, skin) it is reduced by a steroid-5α-reductase to 5α-dihydrotestosterone, the "active androgen". It is a question of definition if we wish to call the secreted and circulating agent "the hormone", or if this term should be used for the substance arising in the target cell and exerting the action. According to the original definition by

Starling (1906), the first alternative would be the appropriate usage. But even in the latter case, it is confusing to term testosterone a "prohormone": This term should be restricted to proteins that serve as precursors of peptide hormones (e.g. proinsulin).

Ecdysone is another example of a steroid hormone converted in most target tissues into a second substance, ecdysterone. Even more complicated is the situation with 1α, 25-dihydroxycholecalciferol where different tissues are involved that are not, *per se*, endocrine glands. A detailed discussion is beyond the scope of this article.

5. Enzymes of Inactivation. For a hormonal control of any physiological function, the active hormone must be either removed from the site of action and/or the circulation, or it must be inactivated. The latter process is exerted mainly by steroid dehydrogenases; they reduce the unsaturated ketone group in ring A with NADH (or NADPH) as hydrogen donor. Though the liver is the main site of steroid hormone metabolism, some inactivation in the target tissue should not be excluded. On the other hand, binding of steroid hormones to proteins may delay their inactivation.

6. Hormone Receptors. It is now generally accepted that the physiological action of hormones is mediated by hormone receptors. In the case of steroid hormones, these receptors are not bound to the membrane; they are soluble proteins occuring within the cell. The generalized picture is that the steroid first binds to the cytosolic receptor. As a result of this interaction, the conformation of the receptor protein is changed, and the receptor-hormone-complex enters the nucleus, as outlined in the left part of Fig. 1. The binding of hormone by receptors is characterized by high affinity, in contrast to the binding by transport proteins. This is discussed in detail in a special chapter in this volume.

7. Induction of Proteins by Steroid Hormones. In the foregoing section, the steroid plays a rather passive part in protein-steroid-interactions. This is reversed in the process to be discussed now, the essential part of steroid hormone action, summarized schematically in Fig. 1.

Evidence for the induction of proteins by steroids came mainly from two lines of research. One was the study of enzymes of amino acid metabolism and gluconeogenesis after cortisol treatment, showing an increase in enzyme activity. The second was the observation that the steroid hormone ecdysone can induce puffs in salivary glands of insects (Chironomus larvae) (Clever and Karlson, 1960). This led us to the hypothesis that steroid hormones may act by retrieval of genetic information in a process comparable to enzyme induction in bacteria as studied by Jacob and Monod in E. coli. This hypothesis, first published in 1961 (Karlson 1961) and later elaborated (Karlson 1963), led to numerous studies on the influence of steroid hormones on mRNA synthesis, corroborating the original concept. Indeed, for a small number of steroid hormones, the mRNA for the specifically induced proteins has been measured and shown to increase after hormone treatment. One of these systems is the induction of uteroglobin by progesterone, which is of special relevance in our context.

This is not the place to go into the details of these studies on steroid hormone action, induction of mRNA and of specific proteins; the reader is referred to recent reviews (Karlson et al. 1975; Karlson 1979). The concept had to be modified in that not the

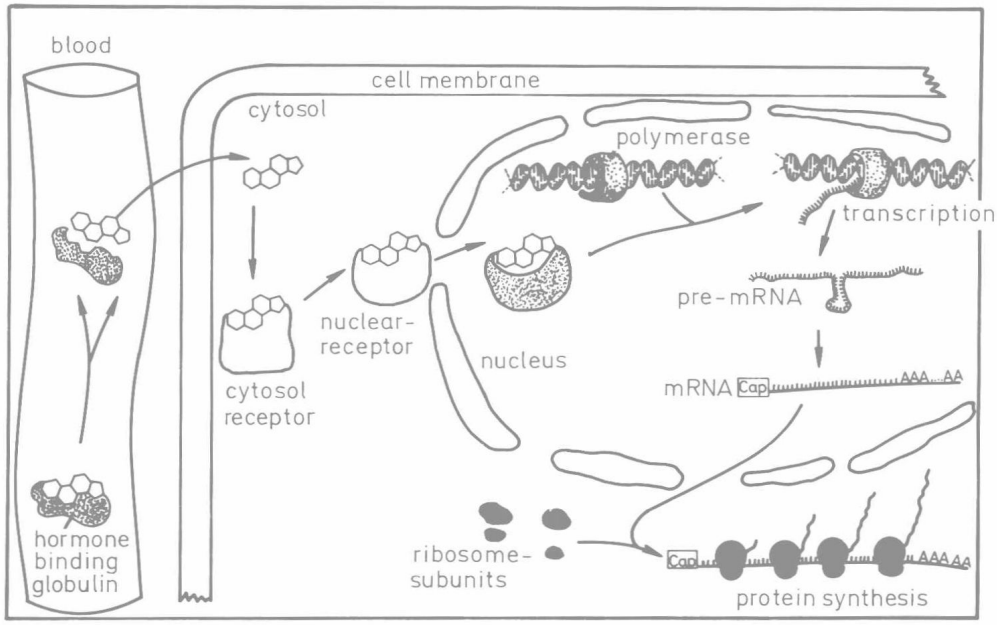

Fig. 1. Schematic representation of the interaction of proteins and steroids in steroid hormone action. In the blood, the steroid hormone is carried by a transport protein (*left*). It enters the cell by diffusion (or perhaps by a carrier transport) as free steroid and is bound, within the cytosol, to the cytosolic receptor. This interaction results in an allosteric conformational change of the protein; the complex is now able to enter the nucleus where it interacts with the chromatin. The nature of this interaction is still unknown, but it results in transcription of the specific gene to be activated by this hormone. Pre-mRNA is produced, it is then processed to yield mRNA that is exported into the cytosol and forms polysomes. The next step is translation of the message into protein. (From Karlson 1979)

"naked steroid", but the steroid-receptor-complex is involved in control of transcription. How exactly this control is exerted, how the special genes to be activated are recognized and how the process of transcription is started is still largely unknown and is under active study in many laboratories.

As result of transcription, pre-mRNA is formed and processed to mRNA which is released into the cytosol where it is translated into protein. There are good indications that some of the many steps in processing and translation are regulated; however, there is no solid evidence, to my knowledge, that steroid hormones participate in the control of posttranscriptional and translational events.

All these protein-steroid-interactions have to be kept in mind when discussing the role of steroid hormones in early pregnancy. The steroid characteristic for early pregnancy is progesterone, one of the prominent proteins is uteroglobin, which is induced by progesterone. Details of their action and interaction are discussed elsewhere in this volume.

It should be pointed out that early pregnancy is characterized by many developmental processes resulting in differentiation. As we understand differentiation, it is the result of differential gene expression. Since steroid-protein-complexes can modify gene expression, I presume that they have much to do with the differentiation of the uterine tissue,

and moreover possibly also the trophoblast and of embryoblast. This system is a challenge to the investigator. Much is known at the morphological and structural level. Also much is known about the general principles of steroid hormone action, as outlined above. But the exact causal relations between steroid (progesterone) and the morphological and functional changes in the uterus remain to be elucidated. A beginning has been made, as is evident from the many contributions presented in this volume. But more information and a lot more understanding of our information is needed.

Among the present problems of mankind, the world-wide control of human population is of vital importance for the survival of the human being. More knowledge about all the details of reproduction in mammals and especially in man can perhaps help to solve our problems. It will not be an easy way; no scientist will expect a "patentrezept" in such a volume. But the information collected here will undoubtedly help others to do the next step.

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Ultrastructure of Trophoblast-Epithelium Relations During Implantation

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In the uterine cavity, the blastocyst usually waits for about a day before it attaches onto the uterine surface and starts invading the endometrium. The preattachment period, however, can be prolonged – this is called delayed implantation. It appears normally among some wild animals but can also be produced experimentally in mice and rats (Bergström 1978).

An experimental delay of implantation is obtained by ovariectomy of a newly mated mouse or rat. While the animal is given progesterone, the blastocyst will slowly attain a low metabolic activity (Bergström 1972a; Menke 1972; Weitlauf 1974), and it will remain in this inactive state until estrogen is given to the animal. A few hours after this injection, the amount of secretion in the uterus increases (Nilsson 1974b; Bergström and Nilsson 1975) and the blastocyst gets metabolically active (Torbit and Weitlauf 1974; Weitlauf and Kiessling 1980). This is named activation of the blastocyst (McLaren 1973; Webb and Surani 1975). Later follows a change in the antigenic properties of the trophoblast (Hakansson et al. 1975; Searle et al. 1976), a decrease of the number of negative surface sites of the blastocyst (Nilsson et al. 1975), and about 24 h after the injection of estrogen, the trophoblast cells have attached onto the uterine surface and begin invading the endometrium (Bergström and Nilsson 1976). Sometimes during this period the blastocyst also initiates early decidual changes in the endometrium (Finn and McLaren 1967; Lundkvist et al. 1979).

1 Blastocysts in Delay

A steady state of delay is reached by the mouse blastocyst about 4 days after the ovariectomy (Bergström 1972a; Naeslund and Lundkvist 1978). Then the blastocyst lies closely surrounded by the endometrium and also the endometrial surfaces of the implantation sites are closely apposed. Practically no luminal secretion is present (Fig. 1).

Ultrastructurally, the most prominent features of the uterine epithelium are apical protrusions and apical vesicles (Warren and Enders 1964; Psychoyos and Mandon 1971; Nilsson 1972). The apical protrusions contain a homogeneous ground cytoplasm and bulge a few micrometer above the microvillous surface (Fig. 2). They are mushroomlike, and since the trophoblast cells lie so close to the uterine surface, the apical protrusions cause craterlike imprints in the trophoblast surface (Bergström 1972a). The apical vesicles are present in a cytoplasmic zone which is separated from the surface membrane by a thin rim of homogeneous ground cytoplasm and from the nuclear level by the Golgi apparatus and a layer of mitochondria (Fig. 3).

Functionally, the blastocyst has a low metabolic activity. This has been shown by various metabolic tests (Menke 1972; Weitlauf 1974; Nilsson et al. 1980) and it is also

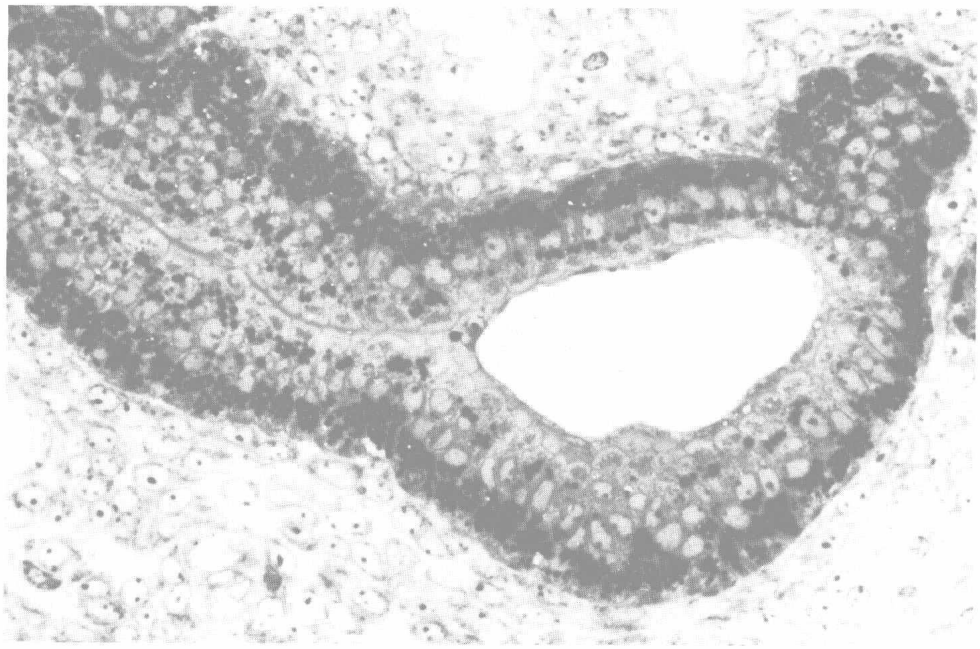

Fig. 1. Blastocyst in uterus from a mouse in delay of implantation. The blastocyst is observed to the *right* and the closed uterine lumen runs from the site of the blastocyst to the *left*. The uterine epithelium contains many dark lipid granules basally and a few lipid granules apically. Light microscopy of Epon-embedded specimen. Mag.400X

visualized in the trophoblast ultrastructure: the ribosomes are scattered randomly and the granular reticulum occurs sparsely (Fig. 4). But although the blastocyst is dormant it does take up nutrients, as judged by the occurrence of endocytotic vesicles (Fig. 5). The endocytotic vesicles are scattered along the borderline and occur also where apical protrusions indent the blastocyst surface. There is thus some form of transport from mother to embryo at these sites.

The apical protrusions themselves are capable of endocytotic activity and have a high capacity for taking up horseradish peroxidase and some stain particles (Enders and Nelson 1973; Parr 1980). But these substances are nonphysiological ones, which might stimulate an endocytosis which is not necessarily relevant for normal conditions.

2 Blastocyst Activation

When estrogen is given, implantation is initiated. Morphologically, the blastocyst gets separated from the uterine mucosa, that is, a uterine secretion appears (Nilsson 1974b; Bergström and Nilsson 1975). One source of the secretion seems to be the apical vesicles of the epithelium. During delay, these vesicles are crowded in an apical region of the cell but are separated from the surface by a layer of ground cytoplasm. A few hours after the estrogen injection, however, they leave their location and empty their – still unknown – contents into the lumen (Nilsson and Lundkvist 1979). At this stage, one

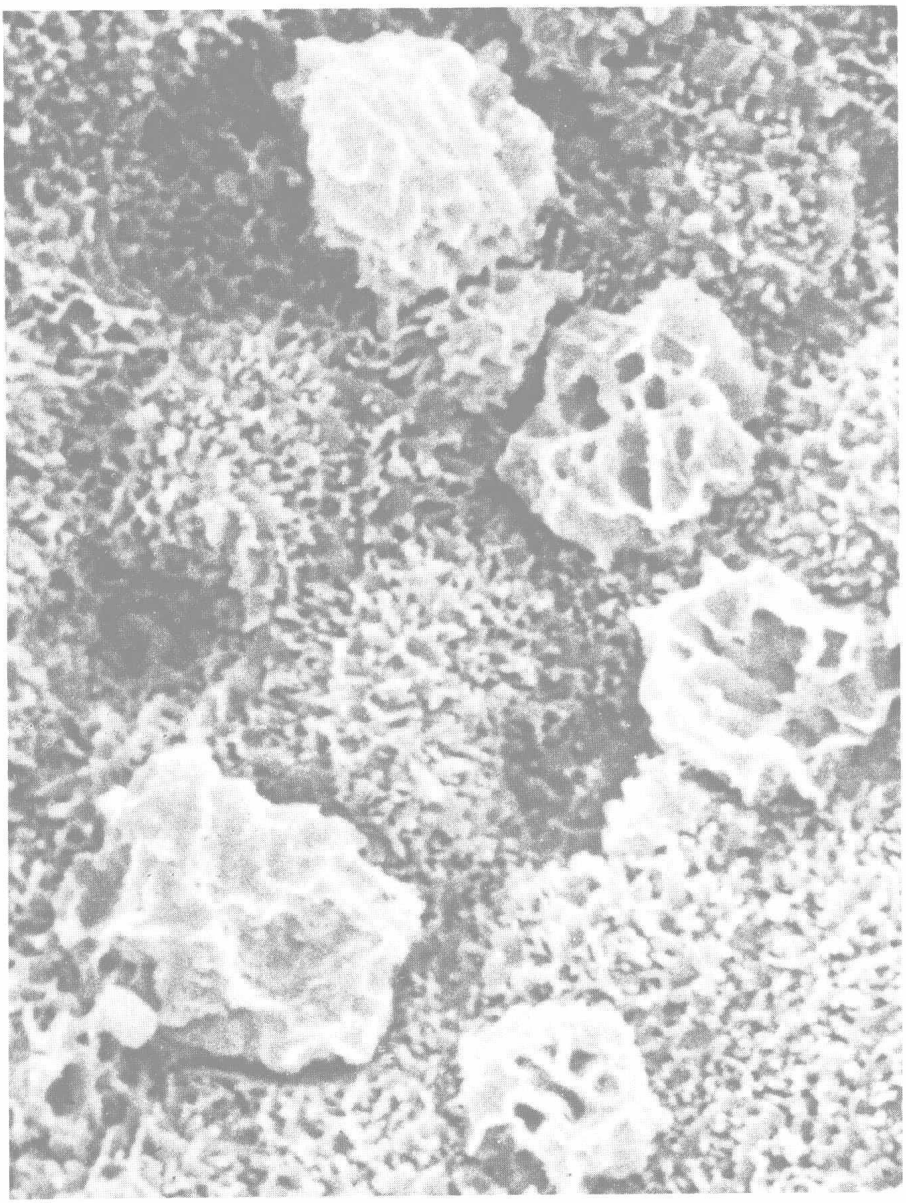

Fig. 2. Luminal surface of uterus from a rat in delay of implantation. Several apical protrusions are noticed among the microvilli of the uterine epithelium. Scanning electron microscopy. Mag.10,000X

important component of the uterine secretion seems to be some type of carbohydrate. Accordingly, as shown by Aitken (Aitken 1976), the amount of fructose increases in the uterine secretion of the roe-deer when the implantatory delay of that animal comes to an end. In the mouse, in-vitro tests have demonstrated that a newly activated blastocyst prefers glucose to fructose and that it also increases its utilization of glucose (Nilsson et al. 1980). Furthermore, it is essential to delete glucose from the culture