4c × 2, 8c × 2) in different ploidy classes predominante in the human myocardium, just as they do in the myocardium of the mouse, rat, guinea pig, and dog [2]. Cells of equal ploidy but with 1, 2, and 4 nuclei did not differ in their protein content. This confirms yet again data showing that binuclear and tetranuclear myocytes are fully adequate analogs of cells of the same ploidy with only one nucleus [2].

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IMMUNOCHEMICAL ANALYSIS OF LECTIN RECEPTORS IN THE STRUCTURE OF FERTILITY α_2-MICROGLOBULIN

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Fertility α_2-microglobulin (FAMG) is a placental protein [2, 7] which is found in a relatively high concentration in extracts of placental tissues and amniotic fluid during the first trimester of pregnancy, and is not detected by immunodiffusion analysis in blood serum in tissue extracts from organs of human adults and fetuses at different times of development [2]. FAMG also is found in the endometrium in the secretory phase [4, 6], and in the seminal vesicles and sperm [4, 9]. This protein has been isolated in a pure form [5] and its physicochemical properties have been studied [3]. FAMG is a glycoprotein containing hexoses (8.7%), hexosamines (6.3%), fucose (0.3%), and sialic acids (2.2%) [7].

Lectins, proteins of animal and plant origin capable of interacting selectively with one or more carbohydrate ligands in the glycoprotein molecule [1], have been used in recent years to study the carbohydrate receptors of glycoproteins [1].

The aim of this investigation was to study interaction between carbohydrate components of FAMG and various lectins, which can shed light on the structure of acceptors of the FAMG molecule and reveal the terminal radicals of the carbohydrates which can bind glycoprotein molecules with lectins.

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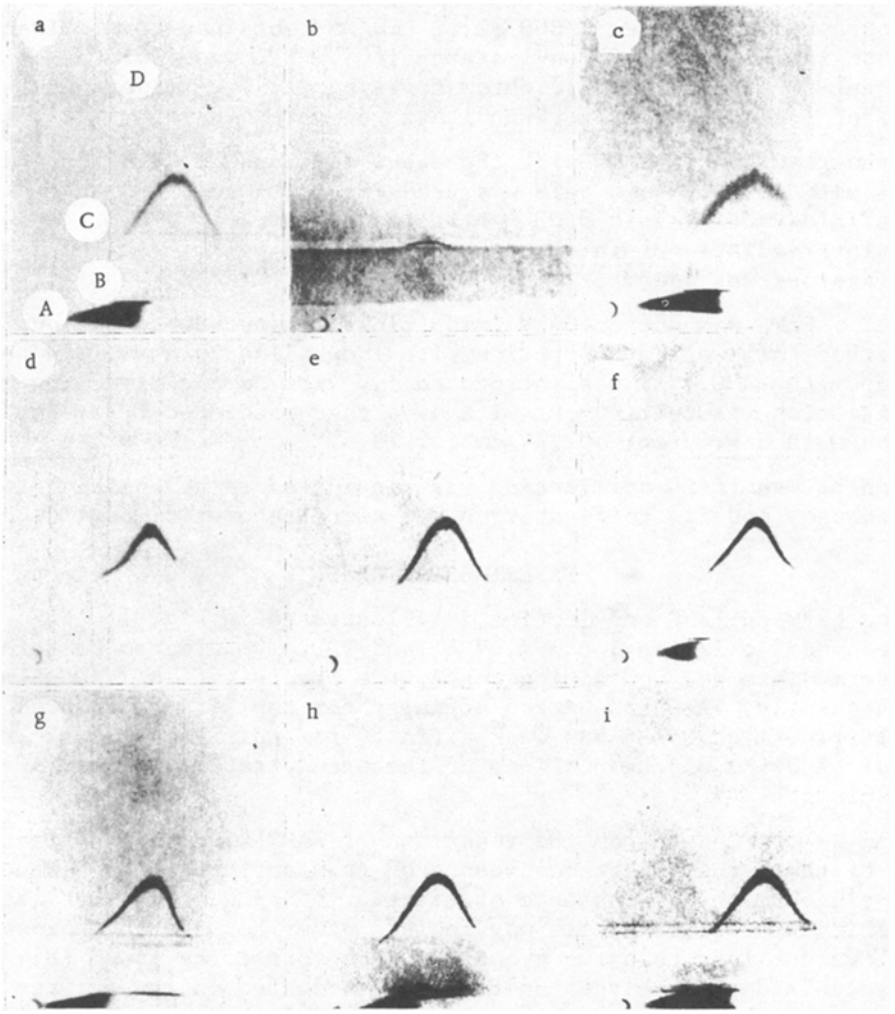

Fig. 1. Interaction of FAMG with lectin. A) Gutter for introducing FAMG preparation; B) intermediate gel containing lectins; C) agarose connecting gel; D) gel containing 5% monospecific antiserum to FAMG. a) Control electrophoresis; b) interaction with con A; c) with PSL; d) with VF1; e) ith HVL; f) with AHF; g) with PHA-P; h) with STL; i) with PEL. In all cases the anode is above.

EXPERIMENTAL METHOD

The following lectins were used to analyze the carbohydrate components of FAMG: concanavalin A (con A), *Pisum sativum* lectin (PSL), *Vicia faba* lectin (VFL), Perch egg lectin (PEL), *Hordeum vulgare* lectin (HVL), *Arachis hypogaea* lectin (AHL), *Solanum tuberosum* lectin (STL), and phytohemagglutin (PLA-P). The first three lectins are mannose-glucose-specific lectins and react with α-D-mannose, α-D-glucose, and N-acetyl-D-glucosamine. PEL reacts only with α-D-fucose. AHL is specific for β-D-galactose. HVL interacts with D-galactosamine and D-glcosamine. PHA-P is specific for N-acetyl-D-galactosamine.

Commercial preparations of con A (Serva, West Germany) and PHA-P (Difco, USA) were used. Other mannose-specific lectins (PSL, VFL) were isolated from the flour of plant seeds by affinity chromatography on Sephadex F-200 [1]. PEL was obtained from extracts of the eggs by affinity chromatography on epoxyfucosyl starch [10]. HVL was isolated by ion-exchange chromatography and gel-penetrating liquid chromatography [12]. AHL was obtained by a combination of methods of precipitation by rivanol and gel-chromatography [11].

To study the reaction of FAMG with the above-mentioned lectins crossed affinity-immunoelectrophoresis with intermediate gels was used [8]. The gel-forming medium was prepared from 1% agarose (Bio-Rad, USA) in 0.02 M barbiturate buffer, pH 8.6. The lectins were introduced into the intermediate gel in a concentration of 5 mg/ml. As the control to the intermediate gel 1% agarose was used.

Antiserum to FAMG was obtained by immunizing rabbits with a semipurified preparation by the standard method followed by absorption with lyophilized plasma from healthy men [2]. Monospecific antiserum to FAMG (5%) was introduced into the second direction gel. Electrophoresis in the first direction was carried out with a voltage gradient of 10 V/cm for 1 h, and in the second direction with a gradient of 2 V/cm for 18 h.

Interaction between FAMG and lectine was considered to be positive if the area of precipitate was reduced, and its configuration and electrophoretic mobility changed [8].

EXPERIMENTAL RESULTS

Interaction between FAMG and lectins is illustrated in Fig. 1. FAMG reacted only with mannose-glucose-specific lectins: con A, VFL, and PSL. On electrophoretic migration of FAMG through the intermediate gel containing con A, the precipitate of FAMG almost completely disappeared. Consequently, FAMG has marked affinity for con A. VFL reduced the area of the precipitate a little, i.e., FAMG has weak affinity for this lectin. The reaction with PSL took the form of blurring of the outlines of the precipitate, possibly due to blocking of antigenic determinants.

Thus it can be concluded from the reactions of FAMG binding with lectins that the terminal radicals of the carbohydrate components of this antigen are α-D-mannose, α-D-glucose, and N-acetyl-D-glucosamine. The absence of reaction of FAMG with other lectins does not rule out the possibility that this protein may include other hexoses and hexosamines, for the method used allows not less than two groups of receptors to be found [8]. Radicals of fucose and other monosaccharides are either uniformly distributed on the surface of the molecule or are not terminal carbohydrates in the composition of the oligosaccharide components of the protein.

The possible nature of the carbohydrate components of FAMG can be deduced from these results. It must be pointed out, however, that the affinity of the carbohydrate receptors for lectins with identical specificity differs. These differences are probably connected not only with affinity of the lectins, but also with the conformity of the carbohydrates composing the oligosaccharide components [1]. The terminal radicals of the carbohydrate components of FAMG are α-D-mannose, α-D-glucose, and N-acetyl-D-glucosamine. The other monosaccharides and hexosamines in the composition of this antigen either lie on the surface of the molecule in the form of single acceptors or are "masked" by other carbohydrate components of the oligosaccharides. The marked affinity of FAMG for con A which we discovered can be used as one stage in the purification of this protein on sorbents with immobilized lectins.

On the basis of these results the preliminary characteristics can be given of the nature of the carbohydrate acceptors for lectins in the structure of FAMG. Step by step degradation of the terminal carbohydrates by the use of appropriate enzymes and subsequent typing of the "unmarked" radicals by lectins will yield a more detailed picture of the structure of the oligosaccharide components of this glycoprotein.

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ULTRASTRUCTURAL CHARACTERISTICS OF EPIDERMOCYTES CULTURED IN MEDIA WITH STANDARD AND REDUCED CALCIUM ION CONCENTRATIONS

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The problem of culture of autologous epithelium for subsequent use of a layer grown in vitro in clinical practice as an alternative method to skin grafting for various skin defects is of great importance [1, 2, 3, 5]. However, there have been few reports of the successful use of autologous skin in clinical practice [6, 7, 9, 10].

One of the basic conditions for culture of epidermocytes is the correct choice of nutrient medium. Reports have recently been published to show that proliferation of cells of the epidermis is accelerated in media with a low (not above 0.8 mM) calcium ion concentration [4, 8, 11]. We have studied ultrastructural changes in epidermocytes cultured in hypocalcium media and have compared them with epidermocytes cultured in media with the standard Ca^{++} concentration.

EXPERIMENTAL METHOD

Epidermocytes were obtained from slices of skin removed with the DRM-60 dermatome from patients during the operation of autodermoplasty (25 cases) and from cadaveric skin (15 cases). The skin graft, taken from different parts of the body, was placed in Eagle's nutrient medium with a high concentration of antibiotics and fungicides (sodium salt of penicillin $1 \cdot 10^4$ U/ml, streptomycin 0.1 g/ml, amphotericin B 500 μg/ml) for 3 to 24 h. The skin graft was then treated with a 0.02% solution of versene for 20 min. The epidermocytes were isolated in 0.25% trypsin solution of 4°C for 18-20 h. The action of trypsin was neutralized by the addition of an equal volume of calf serum. The epidermis was then separated from the dermis. The epidermocytes were washed off by frequent pipetting. The cells were counted in a Goryaev's chamber. The number of cells varied from $1 \cdot 10^5$ to $2.1 \cdot 10^6/cm^2$ of skin graft. The suspension thus obtained was centrifuged at 800 rpm for 10 min. The supernatant was poured off and the residue resuspended in nutrient medium (in hypocalcium medium and, in parallel experiments, in medium with the standard Ca^{++} concentration), and seeded into plastic culture dishes 60 or 100 mm in diameter (Nunc, Coster) with seeding density of $1 \cdot 10^5$-$2.5 \cdot 10^5$ cells/cm^2. The epidermocytes were cultured in a CO_2 incubator (Flow Laboratories) at 37°C, 80% humidity, and in an atmosphere of 95% air + 5% CO_2. The nutrient medium was

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