THE RESULTS of IMBP-ESA joint experiment "Protodyn*"

SOVIET SIDE - Preliminary results

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The objective of experiment

Comparative study of major morpho-physiological characteristics of isolated protoplasts in conditions of space flight (microgravitat ion) and on Earth (normal gravity); growth rates, differentiation and aggregation processes, cell division rate, peculiarities of cell wall formation, determination of its chemical composition, cell ultrastructural rganization, processes of protein synthesis, formation of cytosceleton elements and cell enzymatic activity.

Bioobjects

Freshly isolated seedlings of raps (Brassica napus) and cell culture of carrot (Daucus carota)

Equipment

The experiment has been carried out using special chambers "Type I" constructed by ESA, each of them containing 8 plastic microvolumes - capsules (0.7 ml) hermetically closed with silicone rubber corks in steel rings. Oxygen diffusion into the capsules was ensured, due to semipermeable membrane. Six chambers "Type 1" were used in the experiment which were placed within two containers BB-1M made in USSR.

The conditions of preparing and conducting the experiment

Preliminary stages, such as growing seeds, isolation of protoplasts and preparation of biomaterial were performed in Moscow, at the Institute of Biomedical Problems (Ministry of Public Health of the USSR), together with the specialists from ESA - (Detailed description of the methods for protoplast isolation is presented in the ESA report). Freshly isolated protoplasts in the culture medium were introduced (by 0.7 ml) into the capsules with sterile syringe, 42 h prior to the start of experiment (launch of biosatellite). Each capsule served for definite tasks, in agreement with the programme. Capsules (by 8 pieces) were fixed in the cells of the "Type 1" chambers. Radioactive glucose labeled with C was added to cultivation medium of protoplasts prepared for analysis of composition of newly formed cell walls (the content of N1 chambers in all 8 capsules of experimental and control variants). In turn, the chambers were placed in two containers BB-1M (3 in each). One of the containers (flight variant) was delivered in a transport thermostat (+4C) to the launch site, and 4 h prior to the start was adjusted to the inner surface of technological hatch of the landing apparatus (LA). The other container (control variant) was left in the lab and placed into thermostatic chamber at +4C. Synchronous (control) experiment was initiated 24 h after the biosatellite start, with the aim to have opportunity of correcting the conditions of temperature regime, as corresponds to flight conditions, after having received information from aboard biosatellite using telemetry channels. The start of the biosatellite took place on September 15 at 9.30 a. m.

After landing on September 29 at 7.30, the container BB-1M was dismantled directly at the place of landing, within 1 h. Biomaterial in 4 capsules of the N2 chamber was fixed with 2.5%

glutaraldehide for the purposes of electron microscopic analysis. Then the container with the bioobject was dilevered to Moscow at the Institute of Biomedical Problems, in the transport thermostat (+4 C), after 12 h. The biomaterial being divided between the specialists - the participants of the experiment, following preliminary treatment and microscopic analysis, was taken to various labs for carrying out the studies envisaged by the scientific programme. Analogous operations were conducted using the biomaterial of control after 24 h.

The methods for after-flight studies

Following major components of cell wall polycaccharides were defined: pectin, cellulose, hemicellulose and intracell saccharides. For this purpose, aliquotes were taken from the solutions obtained containing separate fractions of structural polysaccharides, to determine radiosensitivity of the probes. Radioactivity of the specimen was calculated using liquid-cintillatory radiometer "Delta" in the cintillated liquid GS-7. Three probes were taken from each variant. The material for electron microscopic studies was repeatedly fixated with 2.5% glutaraldehide during 24 h. Then the suspension was centrifuged, the pellet placed into 1.2% agarose at 38-40C and impregnated with 1% OsO for 2h. The specimens were dehydrated with ethanole, absolute alcohol and the mixture of alcohol-acetone (1:1) in the order indicated, beginning with 30%, of ethanole, then placed in ethanole-araldite mixture of epoxide resins, kept during 24 h up to complete polymerization.

Ultrathin layers were obtained using microtome LKB and analyzed by electron microscope YEM 100B and YEM-1200EX.

Results and discussion

Quantitative distribution of major components of cell wall in accordance with their radioactivity is presented in Table 1 for control (Earth) and experimental (flight) variants of raps and carrot cell.

Table 1

Radioactivity of several cell wall fractions (mM glucose)

Objects	Variant	Pectin	Hemicellulose	Cellulose	Extracell polysaccha-rides
Raps	Control	2.20+0. 35	0. 41+0.03	1.17+0.15	1.70+0.28
	Flight	2.25+0.32	0.26+0.02	0.54+0.08	1.79+0.25
Carrot	Control	1.25+0.12	0.12+0.02	0.52+0.06	1.05+0.18
	Flight	1.21+0.11	0.076+0.01	0.15+0.02	1.07+0.16

As seen from these data, the content of structural components -cellulose and hemicellulose mostly - in cell walls formed under microgravitation conditions is significantly (about 2 times) lower than that in the control. At the same time, the quantity of non-structureated elements, pectin in particular, as well as initial extracellular polysaccharides is virtually equal both in experimental and in control variants. It should be noted that the regularity observed is characteristic both of raps and carrot cells, differing only in that the total sum of all cell wall components is strongly higher in raps cells than in carrot cell culture. Low content of tructureated elements shown in flight

variants is possibly connected with decrease in the rate of cell wall formation in conditions of microgravitation. One of significant causes for retardation of processes of cell envelope formation is likely to be decrease in general cell metabolic activity. This suggestion is confirmed by the data on decrease in the rate of cell proliferation and biomass accumulation in flight variants. These data are obtained, together with our colleagues from ESA, in the process of preliminary analyses to evaluate biomass by the volume of precipitated after centrifugation suspension of cells formed on the 17th day from isolated protoplasts, in conditions of microgravitation and on Earth. Corresponding data are presented in Table 2.

Table 2

Cell biomass quantity in mg per 1 ml

Objects	Control	Experirnent
Raps	11.2+4.7	9.2+5.8
Carrot	16.4+2.5	9.2+2.2

Electron microscopic analysis of cells formed from isolated protoplasts reveals firstly that great quantity of protoplasts (about 70%) isolated from raps hypocoteles and carrot cell culture regenerate cell envelope. Small portion of protoplasts was being in a state of degradation. Some of them demonstrated clear-cut characters of ageing. In addition, cells embraced by envelope, which though did not start division, were observed.

Such kind of picture was characteristic both of flight and control variants. Consequently, conditions of space flight (microgravitation) are not principal obstacle for regeneration processes, for occurrence of new cell wall, in this case. This notion is supported by the fact that a great number of dividing cells which developed colonial forms (6-9 cells) and were visibly capable of remote proliferation, was detected at the 17th day (the moment of fixation). Colonies consisting of 2-3 cells, evidently stopped in their development, were observed at that.

Great deal of attention was paid to comparative study of cell ultrastructure and its major components» Actively proliferating cells contained dense gialoplasm with numerous polysomes and free ribosomes. Endoplasmatic reticulum is represented by granular short and more long cisterns which often develop aggregates of more or less uniform parallelly situated membranes in separate cell parts.

The nucleus is mainly oval (in non-dividing cells it has cutted blade form), somewhat shifted towards the cell periphery, contains preferentially diffused chromatin and nucleoid with fibrillar and granular complexes.

Plastids are represented mainly by leukoplasts of different shape, size and structure. The protoplasts which regenerated the cell wal1, though still did not start division, or stopped in this process, contain plastids up to 4 mkm in diameter with 2-3 big starch grains. Plastid envelope has sinuous shape, and stroma is of low e1ectron density. Starch hydrolysis is accompanied with increase in the quantity of smaller starch grains takes place in actively proliferating cells. Stroma is electrone-dense.

Mitochondria are mostly of oval shape, mean diameter being 0.3 mkm. Elongated forms of these organells, up to 3 mkm in length, are also encountered. Cristae are clearly seen. However, their quantity decreased significantly in the cells of flight material and e1ectrone-density of mitochondrial matrix is lowered.

Microbodies are of oval form and 0.2-0.3 mkm in size. Dictiosomes consisting usually of 5-6 cisterns produce small formations, about 0.06 mkm in diameter. Separate lipid bits partially or completely surrounded by membrane are noted. Their quantity is some what less in flight variant.

Plasrnalemma is usually situated close to envelope. The cell envelope formed under

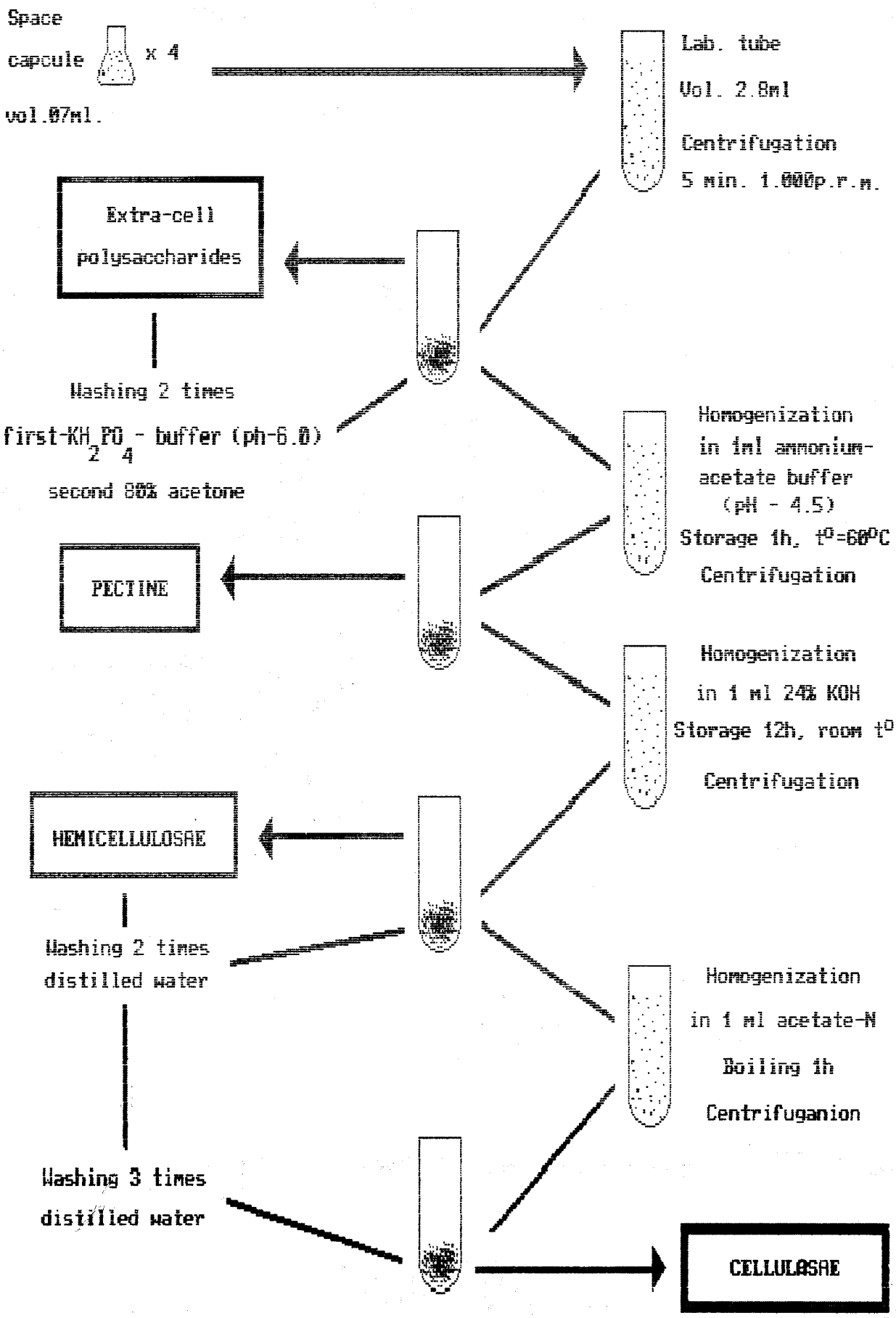
microgravitation is thinner as compared to control, which also points to decrease in the rate of its formation in conditions of microgravitation.

Thus, ultrathin organisation of cells developed from isolated protoplasts under microgravitation and normal gravity force is, in general, similar for raps and carrot. At the same time, the differences mentioned above suggest that the processes of formation of cell elements under microgravitation are slackened in speed.

Analysing the results obtained in that part of studies which was carried out by Soviet specialists, it may be assumed that deviations from the norm of cell physiological status take place in conditions of space flight (microgravitation). To our mind, the changes in metabolic processes under microgravitation resulting from decrease in total energy level are most probable cause for the changes observed.

Taking into account the experimental material accumulated in the field of studies of cell mechanisms underlying influence on the organism of altered gravity force and microgravitation, we can suppose that the given physical factor affects the processes of protoplast development followed by formation of cells, cell aggregates, and their further differentiation. The conditions of space flight, microgravitation, in particular, do not, in fact, interfere with these processes.

More comp1ete picture of cell morphophysio1ogica1 status, when developed in weightlessness and on Earth, may be presented after having discussed the results obtained by ESA specialists.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second 80% acetone
Homogenization
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acetate buffer
(pH - 4.5)
Storage 1h, t°=60°C
Centrifugation
PECTINE
Homogenization
in 1 ml 24% KOH
Storage 12h, room t°
Centrifugation
HEMICELLULOSAE
Washing 2 times
distilled water
Homogenization
in 1 ml acetate-N
Boiling 1h
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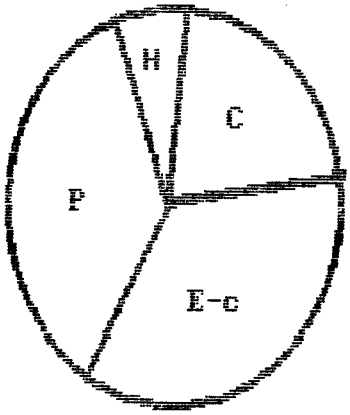
Washing 3 times
distilled water
CELLULASAE

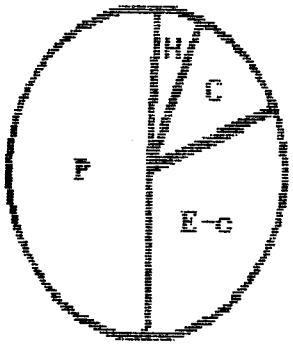
Procedure of cell wall components analysis.

Radioactivity of cell wall several fraction

(mM glucosae)

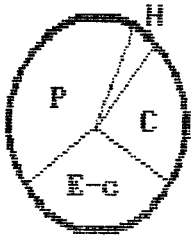


Control

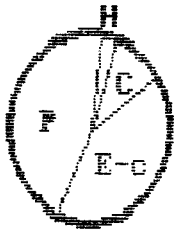


Flight

BRASSICA NAPUS



Control



Flight

DAUCUS CAROTA

P - pectine**;** H - hemicellulosae; C -cellulosae E-c - Extra-cell polysaccharides