CYTO- AND NUCLEOAGGLOMERATION PRODUCED BY SYNCYTIUM-FORMING AGENTS IN MONKEYS

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Spontaneous syncytium-forming agents from monkeys' cells produce cyto- and nucleoagglomeration in cultures of monkey kidney cells, leading to the formation of syncytia and synkaryons. Frequently mitotic divisions without cytotomy are observed in syncytia.

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The ability of syncytium-forming viruses to produce cytoagglomeration has been used in virologic and cytologic investigations. Virologists are interested in syncytium formation as a factor producing fusion of modified infected cells and highly sensitive normally functioning cells, stimulating reproduction of viruses. This is particularly important in the detection of relatively inactive or defective agents or of infinitesimally small numbers of viruses [3, 6, 7].

Cytologists can use syncytium formation as a means of obtaining heterokaryons from cells of various tissues or species of animals, widening the scope of cytogenetic research in these heterogenic synkaryons, enabling hybrid cells to be obtained, and so on [4, 5]. Inactivated Sendai virus is usually used for such investigations [4].

In the present investigation a study was made of cell changes produced by spontaneous syncytium-forming agents from monkeys' cells which can be subcultured only with difficulty.

EXPERIMENTAL METHOD

Three strains of syncytium-forming agents isolated from kidney cell cultures of green guenons [1] were used in the investigation. Numerous attempts to transmit these agents by addition of nutrient medium collected from infected cultures to a culture of monkey kidney cells were unsuccessful. This made it impossible to study the properties of syncytium-forming agents from naturally contaminated cultures. For this reason a modification of the method of subculturing infective material from the nutrient media of infected cultures was developed, using cells of primary and transplanted monkey kidney cultures [1].

Infected and control cultures were fixed after 18, 24, 36, 48, 72, and 96 h with Bouin's and Carnoy's fluids, Shabadash's neutral fixative, 10% formalin solution, and cooled acetone. Material was stained with hematoxylin-eosin and toluidine blue. Nucleic acids were detected by the methods of Brachet and Feulgen and by staining with acridine orange and examination under the luminescence microscope, proteins by treatment with mercuric chloride and bromphenol blue, lipids by Sudan black B, and polysaccharides by Shabadash's method. The method of fluorescent antibodies and treatment with ribonuclease, amylase, and hyaluronidase also were used.

EXPERIMENTAL RESULTS

Specific infection of cells in primary cultures was shown by the development of small syncytia which appeared 21-42 days after explantation. The growth medium usually used was transplantable culture 2607 of kidney cells of green guenons obtained in the Laboratory of Tissue Cultures of the Institute [2]. Syncytia were formed much earlier in subcultures.

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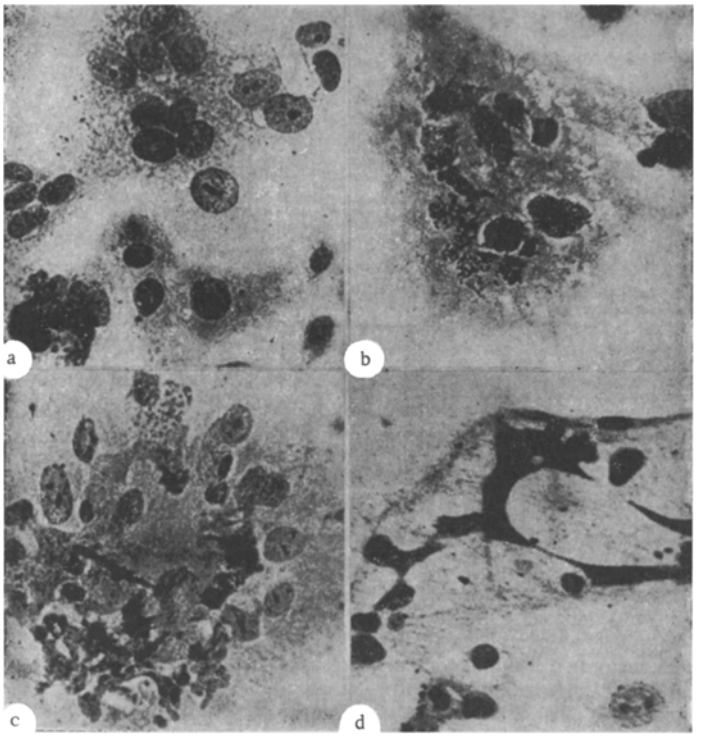

Fig. 1. Syncytium formation (a), mitotic divisions (b, c), and fusion of nuclei (c, d) in syncytia 36-96 h after inoculation of infected culture. Hematoxylin-eosin (a, b, d) and Feulgen reaction (c). 20 × and 40 ×.

After 18 h, before the cells had yet formed a continuous monolayer and were arranged as separate islands, syncytia of various sizes containing from 3-5 to several dozen nuclei could be seen (Fig. 1a). Usually the nuclei were in the center of the syncytia. The cytoplasm of individual syncytia was vacuolated. Vacuoles lay in the center of the syncytia, displacing the nuclei, or around them. Nucleoli in nuclei of the syncytia frequently were enlarged, hyperchromic, and surrounded by a light halo. Chromatin was present as large granules. In some nuclei processes of karyopycnosis and karyolysis were observed. Shrunken and degenerating syncytia also were found.

Mitotic divisions were seen in cells lying next to the syncytia, and in the syncytia themselves chromosomes and figures of different stages of mitosis were formed without cytotomy. In small syncytia usually all the nuclei took part in division, but in large syncytia only some of them did so, and often the chromosomes of different nuclei were at different stages of mitosis (Fig. 1b, c). At these times and later, fusion not only of cells, but also of nuclei could be seen, with the formation of continuous masses and bands (Fig. 1d).

After 24 and 36 h the number of cells on the slide had increased. After 36 h, 78.7 ± 0.6% of the nuclei of the infected culture were in syncytia. The number of syncytia with 6 and more (up to several dozen) nuclei showed a sharp increase (Table 1).

Mitotic activity in the infected culture not only was not lower than in the control, but actually showed a considerable increase (Table 2). Abnormal divisions were rare in the control culture. One chromatid bridge and two multipolar mitoses were observed. In the infected culture, various aberrations were commonly seen among single cells and, in particular, in syncytia. Multipolar mitoses and division of cells and syncytia with disturbance of spindle formation, resembling mitoses taking place under the influence of colchicine (c-mitoses) were observed. The chromosomes were greatly reduced in size and scattered throughout the cytoplasm. Various degrees of contraction, adhesion, and pulverization of the chromosomes, solitary chromatid bridges, and multiple grouping of chromosomes in metaphase also were observed. In the later stages of division, fusion of the newly formed nuclei was found.

TABLE 1. Content of Mono- and Multinuclear Cells in Control and Infected Cultures

Culture and time of observation	Number of multinuclear cells (in %)			Nuclei in multipolar cells (in %)
	with two nuclei	with 3-5 nuclei	with 6 nuclei or more	
	X ± m			
Control 36 h	6.2 ± 0.8	0.75 ± 0.2	0	0.7 ± 0.02
96 h	21 ± 0.9	3.7 ± 0.9	0	2.7 ± 0.1
Infected 36 h	83.5 ± 0.4	58.8 ± 0.6	60.6 ± 8.2	78.7 ± 0.6
96 h	130.5 ± 1.3	118 ± 10.3	239 ± 10.3	86.0 ± 0.3

Note. $P < 0.001$.

TABLE 2. Mitotic Activity in Control and Infected Cultures

Culture and time of observation	Mitotic index (in %) X ± m	Mitoses (in %)					Abnormal mitoses (in %)
		in mononuclear cells				in syncytia	
		P	M	A	T		
Control 36 h	16.75 ± 0.9	13	46	9	32	0	1
96 h	13.3 ± 0.6	12	62	4	22	0	2
Infected 36 h	26.0 ± 0.2	5	55	4	25	11	18
96 h	26.5 ± 0.2	9	41	4	17	29	34

Note. $P < 0.001$.

At later times of observation the processes of monolayer formation and of formation and destruction of syncytia all took place more rapidly. Regression of the syncytia took place both through vacuolation and through karyopycnosis and shrinking of the cytoplasm.

Hypertrophied nucleoli of the syncytium nuclei had an increased RNA and protein content. An increased intensity of reactions for RNA and protein also was observed in parts of the cytoplasm of the syncytia lying next to the nuclei. DNA-containing inclusions were found in the cytoplasm of some syncytia, but they could be formed as a result of phagocytosis of degenerating structures.

The cells, and especially the syncytia, were rich in glycogen. Besides granules of glycogen, a diffuse PAS-positive and metachromatic substance, not glycogen, was also detected in the syncytia. It was almost completely removed by treatment with hyaluronidase. The cytoplasm of the cells and syncytia contained large number of lipid droplets, detected by Sudan black B.

Intranuclear inclusions, characteristic of measles virus, were found in some syncytia. However, the use of the method of fluorescent antibodies and labeled antiserum against measles virus gave negative results.

The results of this investigation show that the modified method of subculture of syncytium-forming agents of low pathogenicity can not only be used for their detection and maintenance, but also to study the processes of cyto- and nucleoagglomeration, the pathology of mitosis, and synkaryon formation.

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