in the cerebellum and medulla suggest that after restoration of the circulation through the common carotid arteries the volume velocity of the blood flow in them rises significantly and marked redistribution of the blood flow takes place between the main vessels of the brain (the common carotid and cerebral arteries). This hypothesis is in agreement with results obtained by Gurvich et al. [4]. Development of a characteristic plateau (Table 1) from 15 to 30 min in the recovery of the blood supply in the postischemic period was evidently due to an increase in vascular resistance, connected with worsening of the circulatory conditions of the arteriolo-capillary network during the postischemic period, to differences in the degree of manifestation of "no reflow" phenomena, and to "late reduction of the cerebral blood flow" in different parts of the rat brain.

Recovery of the blood supply after 60 min of the postischemic period, even though not complete as in the cerebral hemispheres, nevertheless permit complete recovery of the intensity of PL metabolism in those parts in which it was considerably depressed during the period of ischemia.

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REPLICATION OF MOUSE ENCEPHALOMYOCARDITIS VIRUS IN ENUCLEATED KREBS 2 ASCITES CARCINOMA CELLS

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Enucleated Krebs 2 ascites carcinoma cells (cytoplasts) were obtained by centrifuging the cells in a stepwise Ficoll density gradient containing cytochalasin B. The cytoplasts formed opalescent zones in Ficoll solution with a density of 1.037-1.053 g/ml. The cytoplasts did not synthesize RNA, but synthesized cell proteins for a few hours. Mouse encephalomyocarditis virus could replicate in ascites carcinoma cytoplasts, but the yield of virus was only 1/10-1/100 of that in nucleated cells. The decrease in yield was evidently not due to the low efficiency of the early stages of interaction between virus and cytoplasts. Synthesis of cell proteins was inhibited in cytoplasts infected with virus, just as in nucleated cells.

KEY WORDS: Enucleated cells; cytoplasts; mouse encephalomyocarditis virus.

To study the role of the cell nucleus in various biological processes, including replication of animal viruses, it is convenient to use cell preparations from which the nuclei have been removed by special treatment.

Four types of interaction have been described between RNA-containing viruses of animals and enucleated cells (cytoplasts): 1) Sindbis [1, 7], Semliki forest [4] and vesicular stomatitis viruses [5, 13] replicated normally in cytoplasts. 2) Poliomyelitis [4, 10], ECHO and respiratory syncytial [4] viruses and rheovirus could replicate in cytoplasts, but less intensively than in nucleated cells; 3) influenza [5], Japanese encephalitis [7], and Pichinde [1] viruses did not replicate at all; 4) virus-specific macromolecules were synthesized in cytoplasts infected with rabies [13] and measles [6] viruses, but infectious virus was not found.

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TABLE 1. Preparation of Cytoplasts from Krebs 2 Ascites Carcinoma Cells by Centrifugation in Ficoll Density Gradient

Density of Ficoll solution	Centrifugation in Ficoll containing 10 μg cytochalasin B/ml			Centrifugation in Ficoll without cytochalasin B and with corresponding quantity of DMSO		
	percent of cytoplasts	protein content, mg	incorporation of ^{14}C-amino acids, counts·10^{-3}/mg protein	percent of cytoplasts	protein content, mg	incorporation of ^{14}C-amino acids, counts·10^{-3}/mg protein
1.037 1.045 1.053	98.9	0.22	107	36.0	0,49	218
1.060 1.063	94.7	3.15	43	27.8	0,91	49
1.073	49,0	4.64	73	0	5,25	333

In the investigation described below interaction between mouse encephalomyocarditis virus (EMC), which belongs to the Picornaviridae family and the Cardiovirus species, with cytoplasts of Krebs-2 ascites carcinoma cells. The aim was to discover whether this virus can replicate in cytoplasts and, if so, to determine the quantitative characteristics of virus replication and protein synthesis in the infected cytoplasts.

EXPERIMENTAL METHOD

Cytoplasts were obtained by centrifugation of cells previously treated with cytochalasin B, a metabolite of the fungus Helminthosporum dematoideum. Cells grown as a monolayer on cover slips [11,15], in Petri dishes [3], or directly on the surface of the centrifuge tube [2] are most frequently centrifuged. Wigler and Weinstein [14] developed a method of obtaining cytoplasts from mouse lymphoma cells by centrifuging their suspension in a stepwise Ficoll density gradient. To obtain cytoplasts from Krebs 2 ascites carcinoma cells this method was modified.

Krebs 2 ascites carcinoma cells were obtained by weekly passages in female albino mice weighing 20-22 g. The stepwise Ficoll density gradient contained cytochalasin B (10 μg/ml). The original Ficoll solution (50%) was made up in Dulbecco's phosphate buffer, and the cytochalasin B (2 mg/ml) in 95% dimethylsulfoxide (DMSO). The stepwise gradient was composed of Ficoll solutions in saline: 25% (4 ml), 17% (6 ml), 15% (4 ml), 14% (4 ml), 12.5% (6 ml), and 10% (4 ml). The cells ($1.8 \cdot 10^8$) were resuspended in 15% Ficoll solution and added to the stepwise gradient to the solution of the same concentration. The gradient was centrifuged at 22,000 rpm for 45 min on the VAC-601 centrifuge in a 3 × 35 ml rotor. The centrifuge chamber and rotor were first heated to 32°C. After centrifugation the fractions were withdrawn, the Ficoll concentration was determined from the optical rotation, the samples were diluted with saline and centrifuged at 1000 rpm for 10 min, and the residues were washed and resuspended in the same solution. The percentage of cytoplasts was determined in films stained with acridine orange (1 : 1000) in a luminescence microscope.

To determine the concentration of cytoplasmic protein, NP-40 was added (up to 0.5%) to 1 ml of a suspension of nucleated cells or cytoplasts, the mixture was shaken for 5 min at 4°C and centrifuged for 5 min at 2000 rpm, and the supernatant was treated with an equal volume of 1% $HClO_4$, allowed to stand for 30 min at 4°C, and then centrifuged. The residue was dissolved in 0.5 N NaOH overnight and the protein concentration determined by Lowry's method.

The methods of infection with EMC virus, of quantitative determination of the virus, and of determination of the rate of RNA and protein synthesis were described previously [9,12].

Cytochalasin B, actinomycin D, acridione, and DMSO were from "Serva," the Ficoll was from "Pharmacia F.B.," the uridine-^{3}H from the Leningrad "Izotop" Bureau, and the ^{14}C-labeled amino acids from the Radiochemical Centre, Amersham, England.

EXPERIMENTAL RESULTS

After centrifugation of the Krebs 2 ascites carcinoma cell suspension in a stepwise Ficoll density gradient containing cytochalasin B, five or six zones of opalescent material were seen. Most of the cytoplasts were in Ficoll solution with a density of 1.060-1.063 g/ml. Very few nucleated cells were present in this fraction as impurities — not more than one nucleated cell to every 20 cytoplasts (Table 1). After centrifugation of the cell suspension in the stepwise Ficoll density gradient not containing cytochalasin B, several zones of

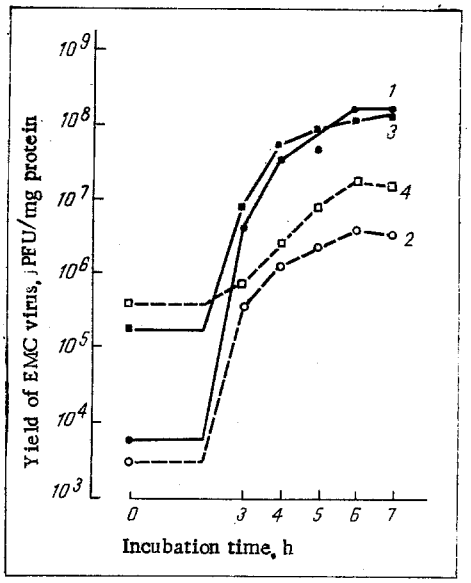

Fig. 1. Replication of EMC virus in nucleated cells and cytoplasts of Krebs 2 ascites carcinoma. 1) Cells infected with EMC virus, incubated at 37°C for 1 h, then kept in Ficoll with cytochalasin B, but not centrifuged (nucleated cells); 2) cells infected with EMC virus, infected at 37°C for 1 h, and centrifuged in Ficoll density gradient with cytochalasin B (cytoplasts); 3) uninfected cells kept in Ficoll with cytochalasin B, not centrifuged, then infected with EMC virus (nucleated cells); 4) uninfected cells centrifuged in Ficoll density gradient with cytochalasin B, zones of cytoplasts drawn off and infected with EMC virus (cytoplasts). Dose of infection $3.3 \cdot 10^5$ to $6.7 \cdot 10^5$ plaque-forming units (PFU)/μg cytoplasmic protein.

fluorescent material also were found. The bulk of the material was located in Ficoll with a density of 1.073 g/ml and contained nucleated cells (Table 1).

Incorporation of uridine-^{3}H into the acid-insoluble fraction, i.e., RNA synthesis, in the cytoplasts amounted to 2-3% of its level in the control cells. Incorporation of uridine-^{3}H into the acid-insoluble fraction of cells treated with actinomycin D (5 μg/ml) was 5% of the level in the untreated cells. In the cytoplasts actinomycin D did not inhibit incorporation of uridine-^{3}H into the acid-insoluble fraction. Thus the synthesis of nuclear RNA was evidently completely absent in the preparations of cytoplasts. Meanwhile the cytoplasts synthesized proteins for at least 4-6 h of their incubation time. Protein synthesis in the cytoplasts amounted to 10-30% of its level in the control cells. Protein synthesis in the cells and cytoplasts was inhibited about equally (by 87-90%) by actidione (100 μg/ml).

TABLE 2. Yield of EMC Virus in Cytoplasts and Nucleated Cells

Material tested	Yield of virus (PFU per sample · 10^{-2}) after infection with			
	virus	virus RNA	virus	virus RNA
	expt. 1		expt. 2	
Nucleated cells	140	360	74	38
Cytoplasts	1,5	30	3	0,2

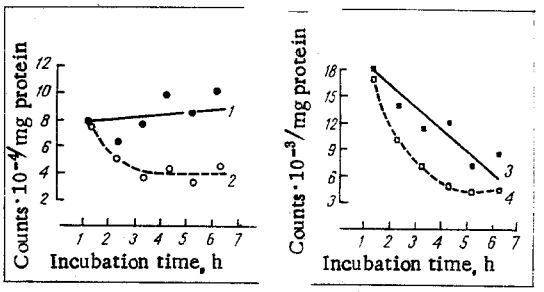

Fig. 2. Rate of protein synthesis in cytoplasts and nucleated cells infected with EMC virus or not infected. 1) Uninfected nucleated cells; 2) infected nucleated cells; 3) uninfected cytoplasts; 4) infected cytoplasts. Nucleated cells kept in 15% Ficoll with cytochalasin B, but not centrifuged. Dose of infection with EMC virus 10 PFU per cell.

EMC virus could replicate in the cytoplast of Krebs 2 ascites carcinoma cells (Fig. 1), but the yield of virus in the cytoplasts, calculated per milligram protein, was only about one-tenth of its yield in the control cells, calculated per milligram of cytoplasmic protein. This decrease in yield was evidently not due to disturbance of the early stages of interaction between virus and cytoplasts. In fact, in cytoplasts obtained from cells infected before and, in which the early stages of interaction took place before removal of the nuclei, the yield of virus also was lower than the yield of virus in nucleated cells (Fig. 1).

Differences in the yields of virus in the nucleated cells and cytoplasts occurred not only when both were infected with virus, but also when infected with virus RNA (Table 2). This, in turn, indicates that the disturbance of replication in the cytoplasts took place at intracellular stages, for the early stages of interaction of virus and virus RNA with the cell are different.

Picornaviruses inhibit synthesis of cell proteins in infected cells. The mechanism of this inhibition is not yet known. It may be that the cells react to virus infection by the formation of a factor which is antagonistic to virus inhibition. The formation of this factor is sensitive to actinomycin D [8]. In that case it can be assumed that the dynamics of inhibition of synthesis of cell proteins in infected cytoplasts will differ from that in nucleated cells.

As will be clear from Fig. 2, rapid inhibition of synthesis of cell proteins took place in nucleated cells and cytoplasts infected with EMC virus. In uninfected cytoplasts there was a gradual decrease in the rate of protein synthesis, but it was less rapid than in the infected cytoplast.

The absence of a cell nucleus thus does not appreciably modify inhibition of synthesis of cell protein by the virus. These findings are in agreement with views on the mechanism of inhibition of protein synthesis in a system of Krebs 2 cells–encephalomyocarditis virus. According to these views, inhibition of cell protein synthesis is the result of competitive substitution of cellular mRNA by virus mRNA.

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DISTURBANCES OF THE Ca^{++} TRANSPORT ENZYME SYSTEM IN MEMBRANES OF THE SARCOPLASMIC RETICULUM CAUSED BY HYDROPEROXIDES OF PHOSPHOLIPIDS AND OF FATTY ACIDS*

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The hydroperoxide (HP) of phosphatidylethanolamine, if added to a suspension of vesicles of the sarcoplasmic reticulum (SR), was shown to have a weak activating effect on Ca-dependent ATPase and to increase the permeability of SR membranes for Ca^{++}, measured during activity of the enzyme. HP of linoleic acid did not affect the parameters of the Ca^{++} transport enzyme system, the activity of Ca^{++}-dependent ATPase, the Ca/ATP ratio, or the rate of outflow of Ca^{++} in SR membranes on account of the low level of its incorporation into SR fragments. It is concluded that among the primary molecular peroxidation products (HP of free fatty acids, HP of phospholipids), induced both in vitro (by the Fe^{++} + ascorbate system) and in vivo (ischemia, avitaminosis-E), only phospholipid HP is an effective modifier of Ca^{++} transport in SR membranes.

KEY WORDS: sarcoplasmic reticulum, hydroperoxides of lipids, membrane permeability, Ca-dependent ATPase.

The development of ischemia is accompanied by accumulation of lipid peroxidation products (LPP) in the membranous structures of the cell [1] and by hydrolysis of phospholipids with the formation of monoacylglycerophosphatides and free polyene fatty acids [8] which, in turn, can undergo autooxidation. Accumulation of LPP is known to lead to an increase in the permeability of artificial and biological membranes for ions and nonelectrolytes [4, 8, 15] and to inhibition of membrane-bound enzymes [2]. Among the many different compounds produced by autooxidation of lipids of biological membranes, the HP of phospholipids in fatty acids, which are primary molecular LPP, occupy a special place: Their ability to intrude into the network of metabolic conversions (through peroxidase reactions) [2] means that the effects of modification of biomembranes by hydroperoxides can be regarded as reversible, whereas in the later stages of lipid peroxidation "irreversible" secondary products (for example, intermolecular polymer cross-linkages), which cannot be utilized by the cell enzyme systems, accumulate. It was shown previously that a consequence of modification of membranes of the sarcoplasmic reticulum (SR) by active forms of oxygen during ischemia of skeletal muscles is an increase in permeability of the SR vesicles for Ca^{++} ions (a decrease in the Ca/ATP ratio) and partial inhibition of Ca^{++}-dependent ATPase [1]; both effects, moreover, are due to the accumulation of LPP. Accordingly, in the investigation described below the action of primary molecular LPP, namely exogenous hydroperoxides of phospholipids and hydroperoxides of free fatty acids, and also unmodified phospholipids and fatty acids, on the parameters of the Ca transport enzyme system, located in SR of skeletal muscles, was studied.

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