EFFECT OF MEDIUM WITH LOW SERUM CONTENT ON PROTEIN SYNTHESIS AND SECRETION AND ON RNA AND DNA SYNTHESIS IN CULTURED SKIN FIBROBLASTS FROM PATIENTS WITH RHEUMATOID ARTHRITIS AND SYSTEMIC SCLERODERMA

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Progressive fibrosis is a characteristic manifestation of system scleroderma (SSD). Most investigators associate the development of fibrosis with disturbance of function of the fibroblasts, expressed in particular as hyperproduction of macromolecules of connective tissue and, more especially, of collagen, by them [3]. Qualitative changes also have been found in proteins contained on the surface of skin fibroblasts of SSD patients [15], and stimulation of collagen synthesis in these fibroblasts on the addition of embryonic calf serum (ECS) to the medium [1, 7]. There is less information on the state of skin fibroblasts of patients with rheumatoid arthritis (RA), although in this disease also increased collagen production has been observed in some strains of these cells [3, 10].

There are good reasons for studying the metabolism of fibroblasts when their proliferation has been arrested by long-term culture in serum-free medium, when biosynthetic processes linked with protein and RNA renewal and catabolic processes [4] are activated, or it has been postulated that activation of skin fibroblasts may be the key process in the pathogenesis of SSD [14], and it probably plays an important role also in the pathogenesis of RA.

In the investigation described below protein synthesis and secretion, RNA and DNA synthesis, and collagenolytic and caseinolytic activity of proteinases secreted by skin fibroblasts of healthy donors (HD) and patients with RA and SSD, and in embryonic fibroblasts (EF) were investigated during long-term (up to 7 days) culture in medium with a low serum concentration (0.1-1%) or in serum-free medium.

EXPERIMENTAL METHOD

The cell strains and methods of culture were described previously [1]. EF (strain LTsCh-814) were obtained from the Institute of Medical Genetics, Academy of Medical Sciences of the USSR. The cultures were studied at the 3rd-10th passage in the stationary phase of growth. Protein, RNA, and DNA synthesis was analyzed with the aid of medium 199, Eagle's medium, and DMEM with the addition of 0.1% bovine serum (BS), of 0.5% ECS, or 0.1% human serum (HS). The medium with low serum content was changed daily. As labeled precursors for the analysis of protein synthesis and secretion, a ^{14}C-protein digest and ^{14}C-valine (Czechoslovakia), ^{14}C, and ^{3}H-fucose (from Amersham International, England) were used, and to measure RNA and DNA synthesis, ^{14}C-uridine (Czechoslovakia) and ^{14}C-thymidine (USSR) respectively, were used. The labeling time, when the synthesis of protein labeled with ^{14}C-amino acids and RNA and DNA synthesis were studied, was 4 h, and for protein labeling with ^{14}C- and ^{3}H-fucose it was 6 h. At the end of the experiment the cells were put on ice and washed with ice-cold Earle's medium. Aliquots of culture medium with labeled protein were centrifuged at 6000 rpm for 10 min to sediment the nonadherent cells, after which 0.1 ml of medium containing 20% BS and an equal volume of 10% TCA was added. The protein residue was dissolved in 0.2 M NaOH, after which the protein was reprecipitated with TCA. Lysis of the cells was carried out as described previously [1] and the acid-insoluble material was precipitated with 10% TCA. The radioactiv-

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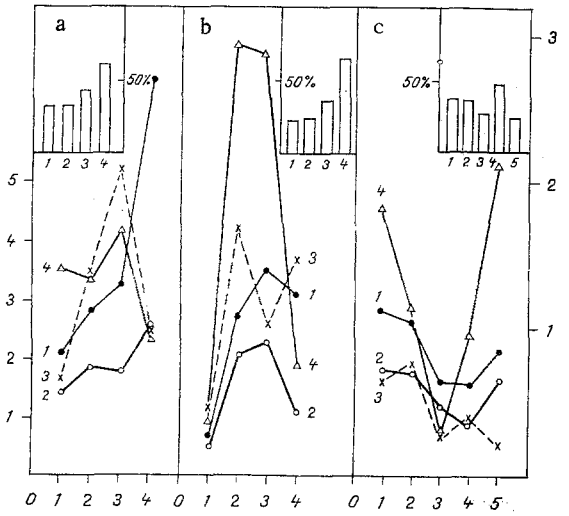

Fig. 1. Protein synthesis (1), quantity of labeled protein remaining in cells (2), and RNA (3) and DNA (4) synthesis by skin fibroblasts of HD (a), by EF (b), and by skin fibroblasts of SSD patients (c) during culture in medium 199 with 0.5% ECS. Abscissa, time (in days); ordinate: on left — incorporation of ^{14}C-valine (in counts × 10^5/min) on right — incorporation of ^{14}C-uridine (in cpm × 10^5/min) and ^{14}C-thymidine (in cpm × 10^4/min). All values calculated per 10^6 cells. Insets: fraction of secreted protein (in % of total quantity of protein synthesized).

ity of the acid-soluble fraction was determined after fixation of the cells with a mixture of ethanol and glacial acetic acid [11].

The collagenolytic and caseinolytic activity in the medium was determined by measuring the degree of hydrolysis of ^{14}C-acetylated collagen [2] from rat skin (10^6-$1.5 \cdot 10^6$ cpm) and of ^{14}C-acetylated casein ($2 \cdot 10^6$-$2.5 \cdot 10^6$ cpm), respectively. Hydrolysis was carried out in 0.01 M Tris-HCl, pH 7.5, containing 1 mM $CaCl_2$, with collagen in a concentration corresponding to 10-20,000 counts per sample, and casein corresponding to 10-30,000 counts per sample. The reaction was stopped by the addition of 10% TCA, and when collagen was used, 0.5% tannin was added to the TCA. Proteolytic activity was calculated from the radioactivity of the supernatant.

The radioactivity of the samples was measured as described previously [1]. The cells were counted in a Goryaev's chamber.

EXPERIMENTAL RESULTS

Transition of skin fibroblasts from HD and EF into a resting state during culture in medium 199 with 0.5% ECS was accompanied by increased synthesis and secretion of protein and an increase in RNA and DNA synthesis on the 2nd-3rd days, especially in EF (Fig. 1a, b). Conversely, synthesis and secretion of protein by skin fibroblasts of SSD patients was reduced under these conditions until the 3rd-5th days of culture (Fig. 1c) and by skin fibroblasts from patients with RA, until the 3rd-4th day (Table 1), but skin fibroblasts from patients with RA, under those conditions, secreted up to 80% of all proteins synthesized de novo, far more than skin fibroblasts from SSD patients. A very high level of protein secretion (up to 98%) was observed during culture of skin fibroblasts from SSD patients in medium DMEM with the addition of a vitamin mixture only (Table 1).

During culture of skin fibroblasts from HD and patients with RA and SSD in medium DMEM with 1% HS an equally high level of protein secretion (80-95%) was observed (Fig. 2a-c), and a virtually equal quantity of labeled proteins remaining within the cells. The increase in protein synthesis by skin fibroblasts of patients with RA and SSD was accompanied by a considerable increase in RNA synthesis, preceded by an increase in DNA synthesis; the second peak of increase of DNA synthesis coincided with the increase in protein and RNA synthesis.

TABLE 1. Protein Synthesis and Secretion in Culture of Skin Fibroblasts from Patients with RA and SSD, Cultured under Different Conditions

Skin fibroblasts	Time of culture, days	Protein synthesized, cpm/10^6 cells		% of secretion
		total	intracellular	
Patient with RA	1	140 624*	108 156	23
Medium 199 + 0.5%	2	503 254	196 094	61
ECS	3	34 130	13 125	61
	4	28 927	5 938	79
Medium MEM + 0.5%	1	1 876 906**	901 656	48
ECS	2	153 779	131 063	14
	3	2 161 434	1 709 484	20
	4	591 325	349 198	40
Patient with SSD	1	242 157***	43 821	81
Medium DMEM + mixture of vitamins	2	98 747	8 115	91
	3	241 979	8 750	96
	4	45 328	685	98
HD	2	77 106***	38 988	49
Medium DMEM + mixture of vitamins	3	57 255	39 806	31
	4	69 786	45 903	34
	5	80 274	58 869	27
	6	58 222	49 835	15
Medium DMEM + mixture of vitamins	2	1 068 277**	422 816	61
	3	333 269	215 498	35
	4	632 961	390 335	38
	5	1 895 664	1 286 708	32
	6	1 772 924	1 301 334	27

Legend. *Incubation with ^{14}C-valine, **) with ^{14}C-proline, ***) with ^{14}C-protein digest.

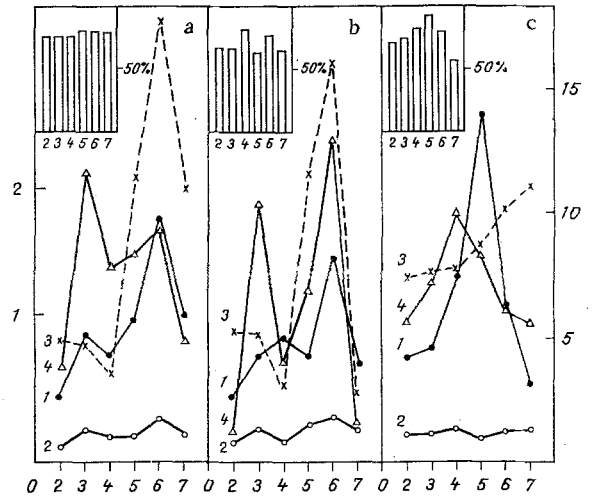

Fig. 2

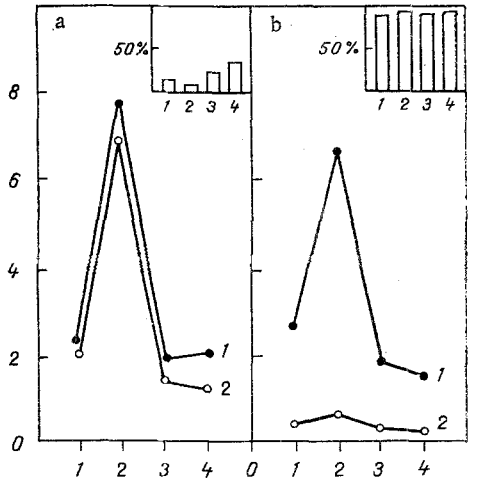

Fig. 3

Fig. 2. Protein synthesis and secretion and RNA and DNA synthesis by skin fibroblasts of patients with RA (a), SSD (b), and HD (c) during culture in medium DMEM with 1% HS. Legend as to Fig. 1 except that incorporation of ^{14}C-thymidine is expressed in counts $\times 10^3$/min.

Fig. 3. Synthesis (1) and amount of glycoproteins remaining in skin fibroblasts (2) from HD (a) and patients with SSD (b) when cultured in medium 199 with 0.5% ECS. Abscissa, time (in days) ordinate, incorporation of ^{3}H- and ^{14}C-fucose (in counts $\times 10^4$/min/10^6 cells). Insets: fraction of secreted glycoproteins in % of total quantity of glycoproteins synthesized.

By the 4th-5th day in medium DMEM without serum, synthesis of ^{14}C-proline-labeled protein showed a sharp increase, and in skin fibroblasts from HD there was a corresponding increase in synthesis and accumulation of labeled intracellular protein; the increase in synthesis of proteins labeled with ^{14}C-protein digest and their accumulation in cells from HD were less marked (Table 1).

Up to 48 and 35% of proteins labeled with ^{14}C-proline were secreted by skin fibroblasts from patients with RA and SSD during culture in medium DMEM without serum and with 0.5% ECS respectively (Table 1). Skin fibroblasts from HD, in the stationary phase of growth, when cultured in the ordinary way secreted only 10% of these proteins, compared with only 7% by skin fibroblasts from patients with SSD.

Synthesis of fucose-labeled glycoproteins reached a maximum on the 2nd day in skin fibroblasts from HD and patients with SSD when cultured in medium 199 with 0.5% ECS, but nearly all the glycoproteins synthesized de novo in skin fibroblasts from SSD patients were secreted into the medium, whereas the fraction of secreted protein in fibroblasts from HD was 10-30% (Fig. 3a, b).

An increase in incorporation of labeled precursors into macromolecules was observed despite their virtually equal contents in the acid-soluble fraction of the cells, analyzed from the 1st through the 6th days of culture.

The collagenolytic and caseinolytic activity in the culture medium of skin fibroblasts of HD and patients with RA when cultured in medium DMEM with 1% HS reached a maximum on the 4th-5th day. The level of collagenolytic activity in medium of fibroblasts from patients with RA was within normal limits or exceeded them by 30-50%, whereas caseinolytic activity was 30-60% below normal, although it still remained at a sufficiently high level on the 6th day of culture.

The high level of protein, RNA, and DNA synthesis observed in skin fibroblasts during long-term culture in serum-free medium was probably due to an increase in the rate of protein and RNA renewal, which may be connected with the fact that these cells need to preserve their liability and proliferative potential [4]. Ability to accumulate protein [6], which cells lose in serum-free medium, may perhaps lead not only to more intensive protein degradation by intracellular proteolytic enzymes [5], but also to more intensive protein secretion, which we observed.

Removal of serum from a medium is known to stop cell proliferation and to stimulate uptake of Ca^{++}, which participates in emission of the mitogenetic signal [9], from the medium [8]. The higher level of DNA synthesis in skin fibroblasts of SSD patients may perhaps be associated with their ability to accumulate higher Ca^{++} concentrations than fibroblasts from HD and patients with RA [12] or with the different quantity of inhibitors of DNA synthesis secreted by the cells into the medium [13].

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