SYNTHESIS OF PLASMA MEMBRANE PROTEINS IN CELLS OF THE REGENERATING LIVER

A. V. Pospelova and N. V. Gorelova

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Protein synthesis was studied by determining incorporation of [^{3}H]glycine in cells of the regenerating rat liver. The rate of incorporation of [^{3}H]glycine into total liver proteins, proteins of the microsomal fraction and of the hyaloplasm, and proteins of the plasma membranes, soluble and insoluble in 0.05 M K_2CO_3 was determined. The rate of incorporation of [^{3}H]glycine into soluble proteins of the plasma membranes reached a maximum 1 h after partial hepatectomy. The maximal rate of synthesis of proteins of the other fractions occurred at the end of the g_1 period. The sharp increase in the rate of synthesis of plasma membrane proteins soluble in 0.05 M K_2CO_3 is evidently one of the earliest biochemical events in cells of the regenerating liver preparing to divide.

KEY WORDS: *plasma membranes; regeneration of the liver; [^{3}H]glycine; protein synthesis.*

Synthesis of RNA, proteins, and other compounds essential for the two main events of the mitotic cycle — DNA replication and mitosis itself — takes place in cells preparing to divide. Intensive study of these processes has enabled a scheme, admittedly far from complete, of the sequence of events culminating in cell division to be drawn up [5]. During the study of the processes preparing the cell for division, the beginning of the prereplicative period is of particular interest. An essential role in the initiation of the cell cycle is evidently played by the state of the cell plasma membranes [3]. However, the metabolism of its components has so far received little study. A few investigations conducted on cell cultures has been devoted to this problem [8, 11, 13]. In the investigation described below changes in the rate of incorporation of a radioactive amino acid into proteins, soluble and insoluble in K_2CO_3, of the plasma membrane proteins of rat liver cells were studied after partial hepatectomy.

EXPERIMENTAL METHODS

Experiments were carried out on male albino rats weighing 170-200 g, on which partial hepatectomy was performed. A mock operation (laparotomy) was performed on the control animals. The animals were killed from 1 to 30 h after the operation. The liver from 8 to 12 partially hepatectomized rats and from 4 to 5 control animals was used in each experiment. [^{3}H]Glycine in a dose of 0.5 μCi/g body weight was injected intraperitoneally into each rat 30 min before sacrifice.

To obtain the fraction of microsomes and hyaloplasm 5 g of liver was homogenized with 3 volumes of 0.001 M $NaHCO_3$ (pH 7.5) in a glass homogenizer with Teflon pestle. The homogenate was centrifuged for 10 min at 1000g. The residue was discarded. The supernatant was diluted with 0.001 M $NaHCO_3$ solution up to 10 volumes relative to the weight of tissue taken and centrifuged for 15 min at 20,000g. The residue was discarded. The supernatant was centrifuged for 100 min at 100,000g. The resulting supernatant was used to determine the radioactivity of the hyaloplasm proteins. The residue was suspended in 1 volume (relative to the weight of the tissue) of 0.001 M $NaHCO_3$ solution and used to determine the radioactivity of proteins of the microsomal fraction.

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TABLE 1. Activity of Na^+-, K^+-, and Mg^{++}-ATPase and of Glucose-6-phosphatase in Plasma Membranes and Homogenate of Rat Liver, μmoles P_i/mg protein/h (mean data)

	Na^+, K^+-ATPase		Mg^{++}-ATPase		Glucose-6-phosphatase	
	membrane	homogenate	membrane	homogenate	membrane	homogenate
Intact liver	2,39 (0,67—4,35) (*n*=7)	0,42 (0,22—0,92) (*n*=7)	12,90 (4.62—22,29) (*n*=7)	3,81 (1,37—7,00) (*n*=7)	2,28 (0,00—4,50) (*n*=8)	3,14 (1,25—4,63) (*n*=8)
Regenerating liver	1,96 (0,64—3,36) (*n*=3)	0,62 (0,37—0,86) (*n*=3)	12,16 (4,34—17,05) (*n*=3)	5,52 (3,60—7,22) (*n*=3)	1,09 (0,00—2,10) (*n*=4)	3,08 (1,33—4,50) (*n*=4)

Note. Limits of variations shown in parentheses; n) number of experiments.

Plasma membranes were isolated by Neville's method [9]. The purity of the isolated membrane preparations was verified by testing for activity of a marker enzyme, Na^+,K^+-ATPase, in it [6]. Activity of Mg^{++}-ATPase [6] and glucose-6-phosphatase [12] also was determined.

Total ATPase activity was measured in medium containing 3 mM ATP, 4 mM $MgCl_2$, 34 mM KCl, 66 mM NaCl, and 25 mM Tris-HCl buffer, pH 7.4.

The activity of Mg^{++}-ATPase was determined in a medium containing 3 mM ATP, 5 mM $MgCl_2$, 100 mM KCl, and 50 mM Tris-HCl buffer at pH 7.2.

The Na^+,K^+-ATPase activity was determined by the difference between ATPase activity in medium containing Mg^{++}, Na^+, and K^+ and the Mg^{++}-ATPase activity. Samples were incubated for 20 min at 37°C.

Glucose-6-phosphatase activity was determined in an incubation mixture consisting of 0.05 M sodium salt of glucose-6-phosphate, 0.1 M acetate buffer, pH 6.0, 0.3% Triton X-100, and 0.1 ml enzyme. The total volume of the incubation mixture was 0.5 ml. The samples were incubated at 37°C for 20 min. The results are given in Table 1. Inorganic phosphorus was determined by the Fiske—Subbarow method [7] and the protein concentration by Lowry's method.

During fractionation of the membrane proteins, a protein soluble in 0.05 M K_2CO_3 was extracted from them [10]. For this purpose the residue of the membranes was suspended in 2-3 ml of 0.05 M K_2CO_3, allowed to stand for 30 min at room temperature, and centrifuged. The supernatant containing proteins soluble in 0.05 M K_2CO_3 was pooled and used to determine radioactivity. The residue was washed with 0.05 M K_2CO_3, suspended in 1-2 ml 0.1 M NaOH, and used to determine the radioactivity of proteins insoluble in 0.05 M K_2CO_3.

To determine radioactivity, an equal volume of 10% TCA was added to all the samples, which were then quickly mixed. The precipitate thus formed was deposited on Millipore filters (Synpor 6, from Chemapol, Czechoslovakia), washed with an equal volume of 5% TCA, and dried. Radioactivity was determined by means of the Mark 2 (Nuclear Chicago) liquid scintillation counter in a toluene—PPO—POPOP system (3.5 g POPOP and 0.1 g PPO to 1 liter toluene).

EXPERIMENTAL RESULTS

The duration of the individual periods of the cell cycle in the regenerating rat liver has been adequately studied. DNA synthesis in the regenerating liver of rats weighing 180-200 g begins 14 h after partial hepatectomy [2]. The period of DNA replication (the S period) lasts 8-10 h. The duration of the postreplicative period (g_2 period) is 3-5 h. The first maximum of mitoses is observed 27-28 h after the operation [1]. Data showing changes in the rate of incorporation of radioactive glycine into proteins of some subcellular fractions of the regenerating liver are illustrated in Figs. 1 and 2.

The rate of incorporation of the amino acid into total liver proteins (Fig. 1) was increased as early as 1 h after hepatectomy and remained high almost throughout the cycle, falling only toward the beginning of the period of maximal mitotic activity of the remaining part of the liver. The rate of synthesis of membrane proteins soluble in 0.05 M K_2CO_3 increased sharply 1 h after partial hepatectomy, then fell, and in subsequent periods of the cycle was only 50-60% above the initial level (Fig. 2).

The rate of synthesis of proteins of the hyaloplasm and microsomal fraction (Fig. 1) and of membrane proteins insoluble in K_2CO_3 (Fig. 2) changed in a different way in the course of the mitotic cycle. Incorporation of labeled amino acids into the proteins of these fractions began to increase immediately after hepatectomy, but the maximal rate of their synthesis, like that of total liver proteins, occurred at the end of the prereplicative period.

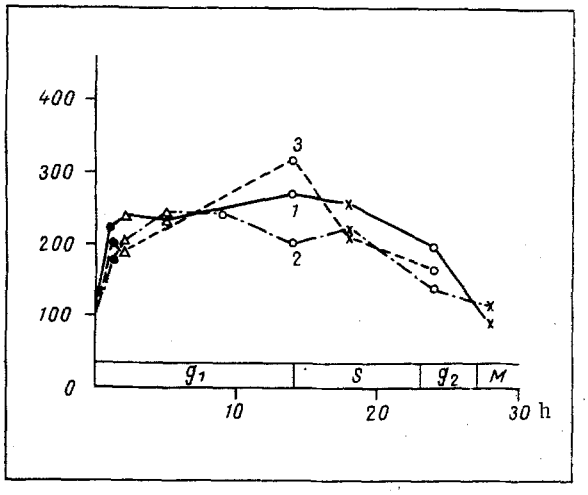

Fig. 1. Rate of incorporation of [^{3}H]-glycine into proteins of homogenate, microsomes, and hyaloplasm of regenerating liver cells of rats at various times after partial hepatectomy. 1) Proteins of homogenate; 2) proteins of microsomes; 3) proteins of hyaloplasm. Points through which curves are drawn are results of one experiment (×) or mean of 2 (■), 3 (▼), or 6 (●) experiments. Abscissa) time after operation, in h, and also periods of mitotic cycle; ordinate) percentages of control.

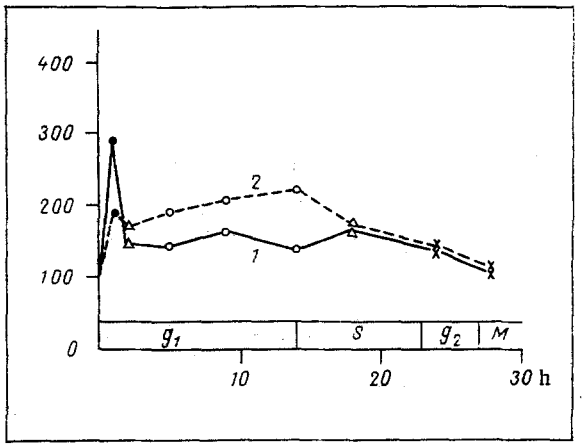

Fig. 2. Rate of incorporation of [^{3}H]-glycine into plasma membrane proteins of cells of regenerating rat liver at various times after partial hepatectomy. 1) Proteins of plasma membranes soluble in 0.05 M K_2CO_3; 2) plasma membrane proteins insoluble in 0.05 M K_2CO_3. Remainder of legend as in Fig. 1.

Gerner et al. [8] studied changes in the rate of synthesis of different components of the plasma membrane in a culture of KB cells synchronized by double thymidine block. These workers found that the rate of incorporation of radioactive amino acid into membrane proteins extractable with 0.5 M NaOH increased after the first synchronous wave of mitoses in the cell culture studied, i.e., in the prereplicative period. The data given above are in agreement with this conclusion.

Changes in the properties of the plasma membrane were observed by workers who studied the proliferation of cells transformed by oncogenic viruses. Terskikh and Malenkov [4], working with a stationary culture of Chinese hamster fibroblasts, found that as early as 15 min after a change of medium, inducing cell proliferation, the permeability of the plasma membrane of the cell induced to divide underwent sharp changes. The results of the present investigation are evidence that the sharp increase in the intensity of synthesis of certain plasma membrane proteins is another of the earliest events taking place in the cell preparing for division.

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MORPHOLOGICAL AND FUNCTIONAL RELATIONS BETWEEN THE THYMUS AND ADRENALS IN INBRED MICE

V. V. Zarudin and V. F. Semenkov

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A morphological investigation was made of the thymus, adrenals, and spleen of mice belonging to the CBA, C57BL/6, and AKR lines, and the immunologic reactivity of the mice to the sex antigen of a skin graft was studied together with determination of the plasma corticosterone level. A narrower cortical layer in the thymus, wide thymus-dependent zones in the spleen, and a higher corticosterone level were observed in CBA mice than in C57BL/6 and AKR mice, and no reaction was found in the former to sex antigen of the skin graft. Adrenalectomy on CBA mice led to a sharp increase in width of the cortex of the thymus, the appearance of numerous lymphatic follicles in the spleen, and the development of an immunologic reaction to sex antigen of the skin graft. In B mice of the CBA line atrophy of the adrenal cortex was observed, with a sharp decrease in the concentration of sudanophilic lipids.

KEY WORDS: *cortex of the thymus; adrenals; B mice; sex antigen.*

Adrenocortical hormones (glucocorticoids) are widely used in clinical and experimental practice to inhibit reactions of tissue incompatibility. Glucocorticoids act on the central organ of immunity — the thymus [7]. The cortex of the thymus contains cortisone-sensitive thymocytes, which on migration into the medullary layer become cortisone-resistant, like peripheral T lymphocytes [10]. It is accordingly interesting to study the effect of adrenocortical function on the thymus and T cell activity detectable by the transplantation immunity test, as well as to determine the morphological and functional state of the adrenals in B mice in the absence of the thymus. The dependence of the immunologic reaction on sex antigen

Departments of Pathological Anatomy and Microbiology, Smolensk Medical Institute.

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