[CANCER RESEARCH 36, 3346-3349, September 1976]

Protein Synthesis in Dextran Sulfate-treated Ascites Tumor Cells[1]

G. David McCoy[2] and Efraim Racker[3]

Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

SUMMARY

Treatment of ascites tumor cells with dextran sulfate resulted in a marked inhibition of the incorporation of [^{14}C]valine into protein in the presence of a high Na^+ medium. Amino acid incorporation was restored after i.p. injection of these cells into mice or by exposure of the cells to ascites fluid *in vitro*. In a medium high in K^+ and low in Na^+, [^{14}C]valine incorporation into protein took place in dextran-treated cells. Rotenone inhibited the reaction, which could be restored by addition of both inorganic phosphate and either glucose or glucose 6-phosphate.

Quercetin, an inhibitor of the Na^+-K^+-ATPase, markedly depressed the incorporation of [^{14}C]valine into protein in intact ascites tumor cells in a high Na^+ medium. There was little or no inhibition of protein synthesis in dextran sulfate-treated cells when tested in a high K^+-low Na^+ medium. These experiments suggest a relationship between protein synthesis and the operation of the membranous Na^+-K^+-ATPase.

INTRODUCTION

It was shown (2) that treatment of ascites tumor cells with dextran sulfate altered the permeability properties of the plasma membrane. Cells thus treated no longer glycolyzed unless P_i and AMP were added from the outside, thus eliminating the requirement for an intracellular regeneration of these cofactors of glycolysis by an ATPase. The altered permeability properties suggested that these cells may be suited for the *in situ* analysis of biosynthetic reactions such as protein or DNA synthesis that require ATP and other impermeant nucleotides, or of ATP-dependent process such as Ca^{2+} translocation in mitochondria. It was shown (2) that, in the presence of rotenone, the uptake of Ca^{2+} into mitochondria can be driven by addition of ATP to dextran sulfate-treated but not to untreated cells. It is the purpose of this paper to describe studies on protein synthesis in dextran sulfate-treated cells that indicate a regulation of this process via the generation of P_i and ADP by the Na^+-K^+-ATPase of the plasma membrane.

[1] Supported by Grants CA-08964 and CA-14454 from the National Cancer Institute, Department of Health, Education and Welfare, and Grant BC-156 from the American Cancer Society.

[2] Present address: American Health Foundation, Valhalla, N. Y. 10595.

[3] To whom requests for reprints should be sent.

Received March 23,1976; accepted June 9, 1976.

MATERIALS AND METHODS

Reagents

Quercetin, AMP, NAD, glucose 6-phosphate, fructose 6-phosphate, rotenone, defatted bovine serum albumin, dextran sulfate 500, and rabbit muscle lactate dehydrogenase were obtained from Sigma Chemical Company, St. Louis, Mo. PPO and POPOP were obtained from New England Nuclear, Boston, Mass. [*U*-^{14}C]Valine (260 mCi/mmole) was purchased from Schwarz/Mann, Orangeburg, N. Y.

Cells

Ehrlich ascites tumor cells were maintained, harvested, and washed as described (2). The medium (Buffer A) used for washing was composed of 50 mM *N*-tris(hydroxymethyl)methylglycine (pH 7.4), 95 mM NaCl, 5 mM KCl, and 2 mM $MgCl_2$. The pH was adjusted to 7.4 by the addition of 5 N NaOH.

Assays

Isoosmolarity was maintained in all experiments involving changes in P_i concentration by addition of equivalent amounts of NaCl.

Glycolysis and Protein. These were measured as described (2).

Protein Synthesis. The rate of *in vitro* protein synthesis in untreated cells was measured in an incubation mixture consisting of Buffer A and 1 mg cell protein in a final volume of 1.0 ml. Other additions were as described in the legends of the figures and tables. With dextran sulfate-treated cells either Buffer A or Buffer B which contained 50 mM *N*-tris(hydroxymethyl)methylglycine (pH 7.4), 95 mM KCl, 5 mM NaCl, and 2 mM $MgCl_2$ (pH adjusted to 7.4 by the addition of 5 N KOH) was used. After 5 min incubation of the cells at 30° in a Dubnoff metabolic shaker, 0.5 μCi of [^{14}C]valine was added, and the incubation was continued for either 15 or 30 min as described in the legends of the figures and tables. At the end of the incubation period, 0.1-ml aliquots of the mixture were removed, placed on 2.5-cm paper filter disc (Whatman No. 3MM), and prepared for liquid scintillation counting by the procedure of Mans and Novelli (1). Processed discs were placed in scintillation vials containing 12 ml of scintillation fluid (5 g of PPO and 0.31 g of POPOP per liter of toluene) and were counted in a Nuclear Chicago Mark I liquid scintillation counter. Incorporation of radioac-

tivity was linear with protein concentration from 0.3 to 3 mg protein per ml and linear with time up to 45 min.

Dextran Sulfate Treatment and Repair

Cells (10 mg/ml) were incubated for 30 min at 4° in Buffer A containing the indicated amounts of dextran sulfate. Cells were then diluted with 10 volumes of Buffer A, centrifuged at 400 × *g* for 10 min, and suspended in Buffer A to give a final protein concentration of about 20 mg/ml.

For repair, dextran sulfate-treated cells were incubated with ascites fluid (5 mg protein) in 1 ml usually for 90 min at 30° or as described in the legends of the charts and tables. Incubations were terminated by dilution with 10 volumes of Buffer A. The cells were centrifuged at 500 × *g* for 10 min and suspended in Buffer A to give a final protein concentration of about 20 mg/ml.

RESULTS

As shown in Chart 1 exposure of ascites tumor cells to increasing amounts of dextran sulfate resulted in a loss of ability to incorporate [^{14}C]valine into protein in a medium that contains high concentrations of Na^+. After treatment with 200 μg dextran sulfate per ml, the ratio of [^{14}C]valine incorporation was less than 20% of that in untreated cells. When dextran-treated cells were injected i.p. into mice and reisolated after 30 or 60 min, [^{14}C]valine incorporation was fully regained (Table 1). It can be seen from Chart 2 that

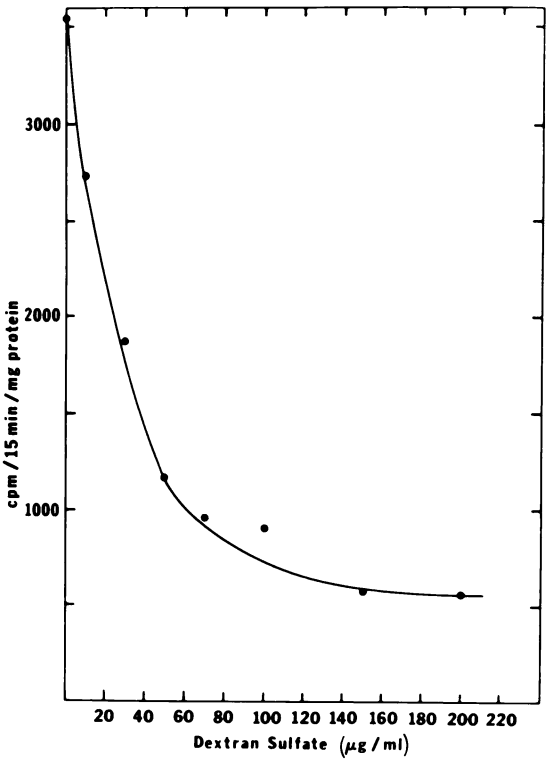

Chart 1. Effect of dextran sulfate treatment on [^{14}C]valine incorporation into proteins of ascites tumor cells. Cells (10 mg/ml) in Buffer A were incubated for 15 min at 4° with the indicated concentrations of dextran sulfate, reisolated, and assayed for [^{14}C]valine incorporation in Buffer A as described under "Materials and Methods."

Table 1

In vivo restoration of [^{14}C]valine incorporation into protein in dextran sulfate-treated ascites tumor cells

Cells (10 mg/ml) were treated with dextran sulfate (200 μg) as described under "Materials and Methods." Five ml of dextran sulfate-treated cells (about 40 mg protein per ml) were injected i.p. into mice. At the indicated times, the cells were harvested, washed, and assayed for [^{14}C]valine incorporation as described under "Materials and Methods."

Time (min)	[^{14}C]Valine incorporation (cpm/15 min/mg protein)
0	321
30	9,185
60	10,402
Untreated cells	8,196

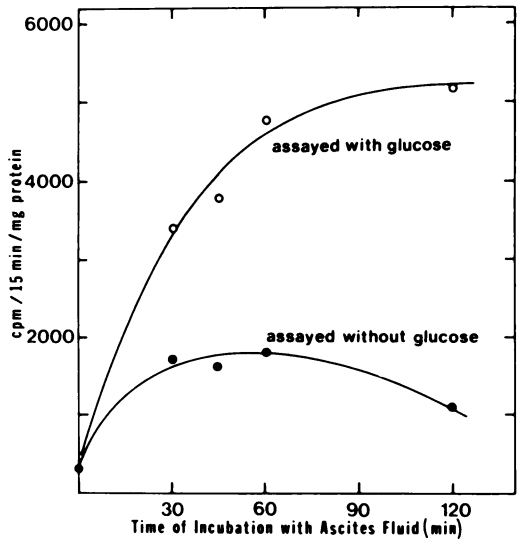

Chart 2. *In vitro* repair of protein synthesis in dextran sulfate-treated cells by ascites fluid. Cells (10 mg/ml) that had been treated with dextran sulfate (200 μg/ml) were incubated with ascites fluid (5 mg protein per ml) for the indicated times, reisolated, resuspended in Buffer A, and assayed for [^{14}C]valine incorporation in Buffer A in the presence and absence of 10 mM glucose as described under "Materials and Methods."

incubation of dextran sulfate-treated cells with ascites fluid *in vitro* restored the ability to incorporate [^{14}C]valine into protein in the presence of a high-Na^+ medium. The repaired cells show a more pronounced dependency on the addition of glucose than do untreated cells, presumably because of depletion of endogenous substrates during treatment and repair.

Chart 3 shows the effect of varying the Na^+/K^+ ratio on [^{14}C]valine incorporation into protein in dextran sulfate-treated cells. When the Na^+/K^+ ratio reflected the normal intracellular ionic environment, [^{14}C]valine incorporation was markedly increased. These data suggest that the inhibition of protein synthesis in cells treated with dextran sulfate is caused largely by the loss of intracellular potassium.

Rotenone, a specific inhibitor of mitochondrial NAD-linked oxidations, inhibited [^{14}C]valine incorporation in both untreated and dextran sulfate-treated cells (Table 2). In untreated cells, the inhibition was reversed by the addition of glucose. Reversal of the rotenone inhibition in dextran sulfate-treated cells required the addition of both P_i and glucose. Chart 4 shows that glucose-6-P substituted for

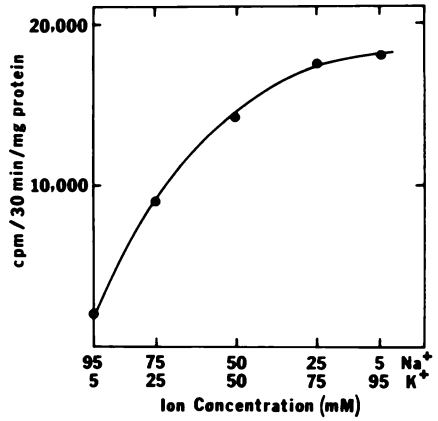

Chart 3. Effect of Na^+/K^+ ratio on protein synthesis in dextran sulfate-treated cells. Cells that were treated with dextran sulfate (200 μg/ml) were reisolated and assayed for [^{14}C]valine incorporation in a medium containing: 50 mM *N*-tris(hydroxymethyl)methylglycine-Tris (pH 7.4), 2 mM $MgCl_2$, and the indicated concentrations of NaCl and KCl. Assays were performed as described under "Materials and Methods."

Table 2

Reversal of the rotenone inhibition of protein synthesis in untreated and dextran sulfate-treated cells by glucose

Treatment of cells with dextran sulfate (500 μg/ml) and assay for [^{14}C]valine incorporation were as described under "Materials and Methods." Both cell preparations were incubated for 5 min at 30° with the indicated additions prior to the addition of isotope.

Additions	[^{14}C]Valine incorporation (cpm/30 min/mg protein)
Untreated cells	
None	29,650
+ Rotenone (0.1 μg/ml)	850
+ Glucose (10 mM)	42,900
+ Rotenone + glucose	42,070
Dextran sulfate treated	
None	12,520
+ Rotenone (0.1 μg/ml)	1,120
+ Rotenone + P_i (50 mM)	1,070
+ Rotenone + glucose (10 mM)	1,610
+ Rotenone + glucose + P_i	13,900

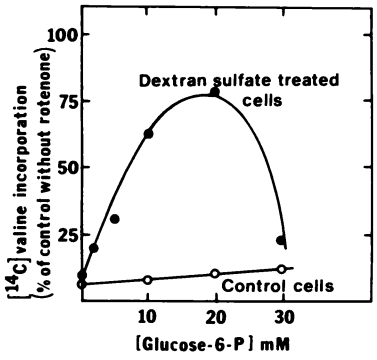

Chart 4. Protein synthesis in untreated and dextran sulfate-treated cells in the presence of glucose 6-phosphate. Untreated cells were assayed for [^{14}C]valine incorporation in Buffer A in the presence of rotenone (0.1 μg/ml) and the indicated concentrations of glucose-6-P. The rate of [^{14}C]valine incorporation in the absence of rotenone was 26,790 cpm/30 min/mg protein. Cells treated with dextran sulfate (500 μg/ml) were assayed for [^{14}C]valine incorporation in Buffer B in the presence of rotenone (0.1 mg/ml), 50 mM potassium phosphate, and the indicated concentrations of glucose-6-P. The rate of [^{14}C]valine incorporation in the absence of rotenone was 12,101 cpm/30 min/mg protein.

glucose in the reversal of the rotenone inhibition in dextran sulfate-treated but not in untreated cells. Relatively high concentrations of dextran sulfate (500 μg/ml) were used in these experiments. This was to ensure rapid permeation of glucose-6-P which is an impermeant ion in untreated ascites tumor cells.

At high concentrations of glucose-6-P [^{14}C]valine incorporation was markedly inhibited (Chart 4). Control cells that had not been treated with dextran sulfate did not significantly incorporate [^{14}C]valine into protein in the presence of rotenone, and addition of glucose-6-P stimulated only slightly at high concentrations. An analysis of the inhibition of [^{14}C]valine incorporation by glucose-6-P revealed that glucose-6-P at high concentrations also inhibited lactate production (Chart 5). Since fructose-6-P did not inhibit at similar concentrations, it seems likely that the point of inhibition is the isomerization of glucose-6-P to fructose-6-P which is known to be sensitive to products of glucose-6-P oxidation via the pentose phosphate cycle. Whether the inhibition of protein synthesis by glucose-6-P can be directly correlated to the lowered rate of glycolysis or is caused by some other mechanism associated with the high glucose-6-P concentrations remains to be elucidated.

Previous reports from our laboratory have shown that the bioflavonoid quercetin is a potent inhibitor of glycolysis in several cell lines (4). It was also shown that, in cells which have been treated with dextran sulfate, glycolysis is no longer inhibited by quercetin (2). The data in Table 3 show that in untreated cells both glycolysis and [^{14}C]valine incorporation into protein were strongly inhibited by low concentrations of quercetin. In dextran sulfate-treated cells tested at high K^+-low Na^+ concentrations, neither [^{14}C]valine incorporation nor glycolysis was inhibited at these concentrations of quercetin.

DISCUSSION

Our studies have shown that exposure of ascites tumor cells to dextran sulfate results in inhibition of respiration, glycolysis, protein synthesis, Rb^+ uptake, stimulation of ATP-dependent Ca^{2+} uptake, and enhanced permeability to

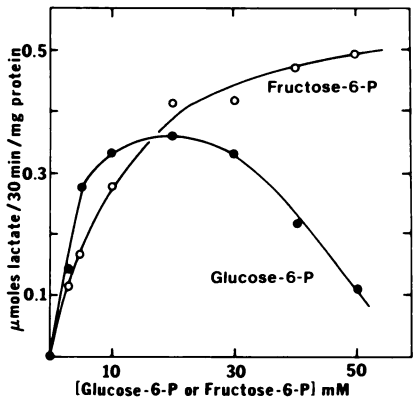

Chart 5. Lactate production in dextran sulfate-treated cells. Cells that had been exposed to dextran sulfate (500 μg/ml) were incubated with the indicated amounts of either glucose-6-P or fructose-6-P in the presence of 5 mM AMP and 50 mM NaP_i and assayed for lactate formation as described in "Materials and Methods."

Table 3

Effect of quercetin on glycolysis and protein synthesis in untreated and dextran sulfate-treated cells

Ascites tumor cells were treated with dextran sulfate (500 μg/ml) as described under "Materials and Methods." Glycolysis in untreated cells was performed in Buffer A. Glycolysis in dextran sulfate-treated cells was performed in Buffer B containing 50 mM potassium phosphate. Both treated and untreated cells were incubated for 5 min at 30° with the indicated additions prior to the addition of glucose. Protein synthesis in untreated cells was performed in Buffer A. In dextran sulfate-treated cells, assays were performed in Buffer B containing 50 mM potassium phosphate. Both treated and untreated cells were incubated for 5 min at 30° prior to the simultaneous addition of glucose and [^{14}C]valine.

	Glycolysis (μmoles lactate/30 min/mg protein)		[^{14}C]Valine incorporation (cpm/30 min/mg protein)	
	Untreated cells	Dextran sulfate-treated cells	Untreated cells	Dextran sulfate-treated cells
− Quercetin	0.24	0.37	55,160	29,170
+ Quercetin (10 μg/ml)	0.08	0.39	19,087	28,730

the dye erythrosin B. The inhibited metabolic functions were restored by supplying the appropriate ionic environment and cofactors. In the case of glycolysis, AMP and P_i were required; in the case of protein synthesis, a suitable K^+/Na^+ ratio was needed. The inhibition of [^{14}C]valine incorporation into protein in dextran sulfate-treated cells was not surprising, since it is well known that protein synthesis in cell-free systems requires K^+. Also expected was the inhibition of this process by rotenone, which interferes with mitochondrial respiration and thereby blocks the generation of ATP required for protein synthesis. However, unexpected was the absence of [^{14}C]valine incorporation into protein in dextran-treated cells in the presence of glucose (Table 3) and the dependency of the process on addition of P_i, since there is sufficient residual phosphate present in dextran sulfate-treated cells to support respiration. The virtually complete dependency on added P_i is probably partly the result of accumulation of phosphorylated intermediates of glycolysis in the presence of glucose which results in the depletion of residual intracellular P_i and nucleoside triphosphates. On the other hand, the possibility that P_i either directly or indirectly influences protein synthesis is attractive in view of our observations that inhibitors of the Na^+-K^+-ATPase affect [^{14}C]valine incorporation into protein as described in this paper. Previous reports on the inhibition of protein synthesis by ouabain (3) are in line with our observations.

The effect of quercetin on [^{14}C]valine incorporation in intact cells is particularly significant because of the control experiments that show no inhibition of either glycolysis or [^{14}C]valine incorporation in dextran sulfate-treated cells. It thus seems unlikely that the primary effect of quercetin is on either a glycolytic enzyme or on an enzyme involved in protein synthesis. We therefore propose that its effect is indirect, via an inhibition of the Na^+-K^+ ATPase, which controls the intracellular concentration of P_i and adenine nucleotides.

These experiments serve to demonstrate the usefulness of dextran sulfate-treated cells for the analysis of biosynthetic process under conditions somewhat more physiological than those in reconstituted enzyme systems.

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