

ALTERATIONS OF GENE EXPRESSION IN NOVIKOFF HEPATOMA
CELLS INDUCED BY A FACTOR IN HUMAN URINE

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SUMMARY: Proteins and protein subunits from Novikoff hepatoma cells have been mapped by two-dimensional polyacrylamide gel electrophoresis utilizing the BASO-DALT system to resolve the basic proteins. Utilizing this technique, it has been demonstrated that human urine contains proteins that retain biological activity and can stimulate synthesis of several new proteins in neoplastic cells. This stimulatory activity has been detected in urine from cancer patients and normal individuals.

INTRODUCTION

It has been repeatedly proposed that many of the structural genes of eucaryotic cells are switched on and off in batteries or sets during differentiation (1). One approach to identify these sets involves the careful indexing of two-dimensional electrophoretic maps from all cell lines during each stage of differentiation in one organism, which is indeed a formidable task. An alternative approach is to seek factors that trigger the expression of individual gene sets in cultured cells. It has been reported that normal human urine contains nondialyzable factor(s) that can inhibit DNA synthesis in transformed cells (2, 3) while urine from muscular dystrophy patients has been shown to inhibit the growth of chick embryo fibroblasts (4, 5). In this paper we show that nondialyzable factor(s) in normal human urine can switch on the synthesis of certain basic proteins in cultured Novikoff Hepatoma cells. Stimulatory activity has been detected in urine from both cancer patients and normal individuals. Cellular proteins were analyzed by two-dimensional polyacrylamide gel electrophoresis, in

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which the first dimension (non-equilibrium) separation (6, 7) is based on charge variation, while the second dimension (SDS electrophoresis) separates proteins on the basis of their molecular weight (8, 9, 10).

MATERIALS AND METHODS

Nondialyzable constituents of urine from normal adults and cancer patients were concentrated as previously described (11), and added to cultures of Novikoff hepatoma cells in amounts of up to 1 mg of urine concentrate per 4×10^6 cells. Cellular proteins were labeled with ^{35}S -methionine by culturing the cells for 48 hours in Iscove's serum-free medium 479 (12) without cold methionine. Both the ^{35}S -methionine and the urine concentrate were added at the initiation of the cultures. At the end of the labeling period the cells were harvested and washed in Dulbecco's modified MEM, and the cell pellets were solubilized in a mixture of phosphatidyl choline and urea as previously described (7). Solubilized samples were centrifuged for 1 hour at $100,000 \times g$ in specially adapted microfuge tubes to pellet high molecular weight material such as intact DNA. The soluble and solubilized cell proteins were analyzed by high-resolution electrophoresis using the BASO-DALT system (7), which is a variation of the NEPHGE method of O'Farrell et al. (6). Gels were fixed, dried, and autoradiographed for 2 weeks as described (13). Note that the gels illustrated are oriented with the basic end of the focusing (first dimension) to the right and the acidic end to the left.

RESULTS AND DISCUSSION

ISO-DALT separations (9, 10) of the acidic and neutral proteins from Novikoff hepatoma cells did not show any differences in the two-dimensional patterns before and after exposure of the cells to urine concentrates from a variety of sources. However, the BASO-DALT procedure (7), which separates the more basic cellular proteins, revealed seven spots in hepatoma cells exposed to urine concentrates that were not visible in the untreated cells (Fig. 1). The four major proteins of the seven revealed after exposure to urine are noted by arrows in Figs. 1 and 2. All seven are elicited in cultures exposed to urine concentrates, regardless of the patient's health. Thus, urines from patients with multiple myeloma (Fig. 1D), lung tumor, or bladder cancer were able to elicit the same seven spots as obtained from normal urine concentrates. Two-dimensional electrophoretic maps from cells cultured with serum from the normal male or female urine

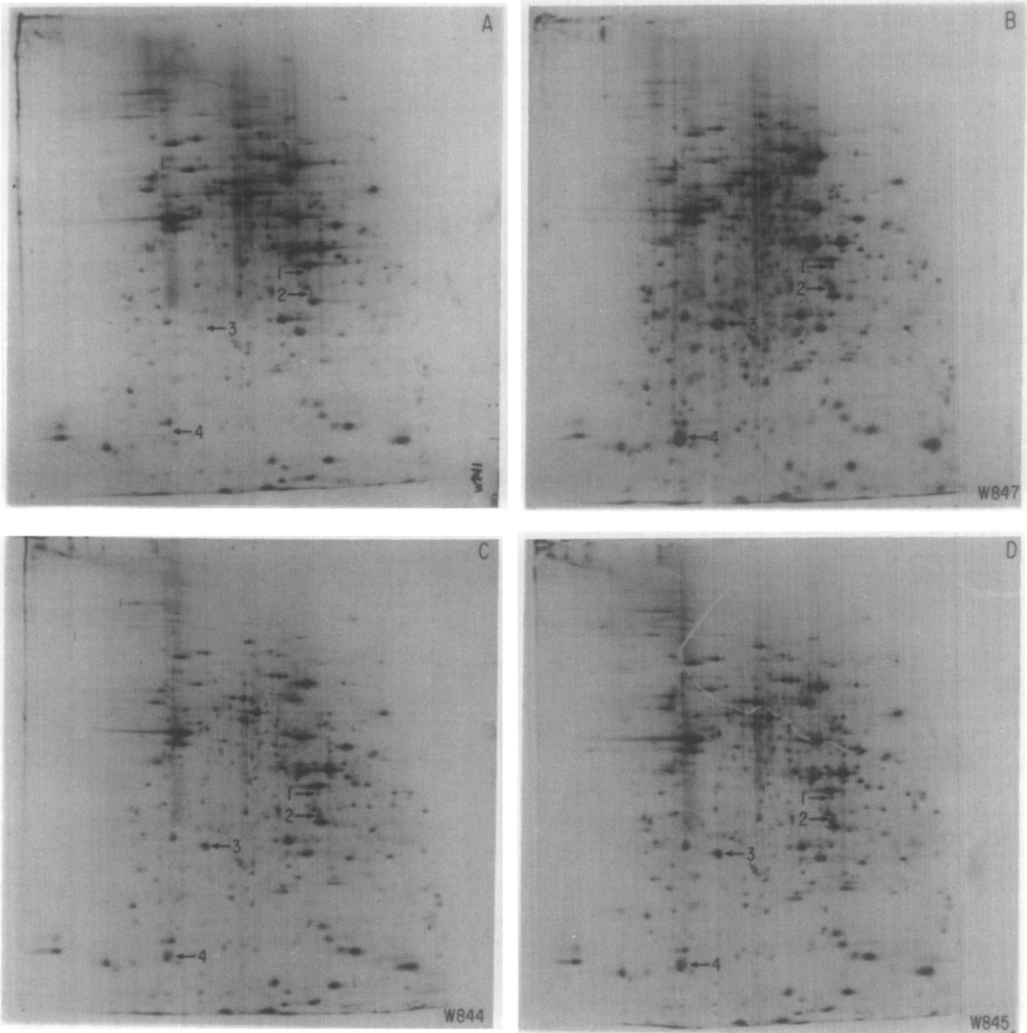


Fig. 1. Patterns of Novikoff hepatoma cells from two-dimensional (BASO-DALT) gel autoradiographs. Samples were electrophoresed for 4000 volt-hours using Brinkman 2 - 11 ampholytes as described (7). Cells (4×10^6) were cultured without urinary proteins (A) or with 1 mg protein from male urine (B), female urine (C), or urine from multiple myeloma patient (D). The arrows indicate four of the new spots found in the cells cultured with urine.

donors did not stimulate production of these new spots, suggesting that the active factor(s) were not present in appreciable concentration in the serum. We have not seen any significant difference in the relative intensity of the seven spots in cells treated with either normal or cancer patient urine preparations.

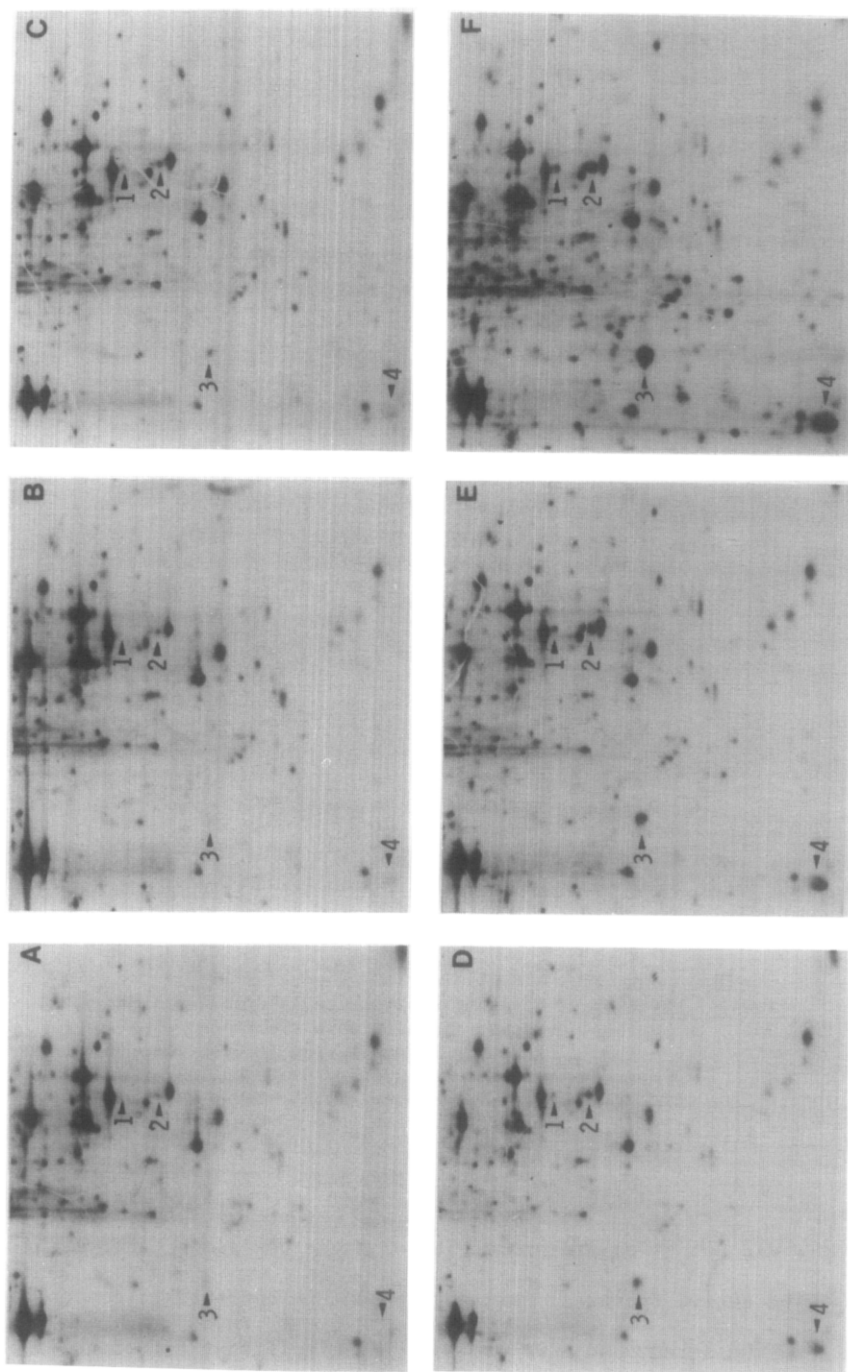


Fig. 2. Center sections taken from several BASO-DALT autoradiographs of Novikoff hepatoma cells. The cells were cultured with normal male urine at the following concentrations: (A) control without urine, (B) 0.0001 mg, (C) 0.001 mg, (D) 0.01 mg, (E) 0.1 mg, and (F) 1.0 mg. Cell samples were pre-
pared and run as described in Fig. 1. Proteins 1-4 are the same as in Figure 1.

The effect was dependent on the amount of urine concentrate added to the cultured Novikoff cells (Fig. 2). The density of all seven spots appeared to increase in proportion to the amount of urine concentrate added to the cells, regardless of the urine source. This suggests, but does not prove, that the seven proteins are members of one set under the control of a single mechanism. At protein concentrations below 10 μg per 4×10^6 cells [as assessed by the Coomassie brilliant blue assay (14)] the activity is lost.

These results support the view (3, 4) that some neoplastic cell lines may be selectively sensitive to a nondialyzable (11) factor(s) found in human urine. Whether this factor is a protein, a small peptide, a large polysaccharide, or a low molecular weight compound, such as a hormone that is tightly bound to a protein, remains to be determined. These observations are of special interest because unpublished experiments conducted in our laboratory have demonstrated that a variety of chemical inhibitors and effectors of cell functions do not have a direct effect on the pattern of protein synthesis as assessed by two-dimensional electrophoresis. We have recently published high-resolution electrophoretic patterns of human urinary proteins from a variety of sources (15), and hopefully the spot or spots responsible for this activity can be located and identified in these patterns.

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