ABSTRACT

Two-dimensional gel electrophoresis was used to detect [35S]methionine-labeled newly synthesized proteins from corticosteroid-sensitive and corticosteroid-resistant P1798 mouse lymphosarcoma cells. When the protein patterns from corticosteroid-sensitive cells are compared with those from highly resistant cells, they are very similar except for seven changes. One of the most obvious changes is the appearance of a new protein with a molecular weight of about 36,000 in highly resistant cells. There are also four other proteins that increase in the resistant state, as well as two that decrease. The control experiments indicate that protein differences are present to approximately the same degree when the sensitive tumor is carried in adrenalectomized animals, suggesting that the protein differences are not induced by endogenous glucocorticoids. Other experiments show that the protein differences are neither components of the small amounts of fibrous tissue that are associated with the cells nor components of blood. These results suggest that resistance of the tumor cells to glucocorticoid killing in vivo could be related to the relative rates of synthesis of a few proteins.

INTRODUCTION

High levels of glucocorticoid hormones produce lethal effects in many lymphoid cells. Such hormone-induced cell killing has provided effective chemotherapy in a variety of malignant states. However, the usefulness of such therapy is often short-lived because of the eventual emergence of cells resistant to glucocorticoid killing. In certain model cell culture lines, such as S-49 cells, the mechanism leading to the resistant state appears to be an absence of, or a defect in, hormone receptors or receptor-related phenomena (16). Even in the transplantable P1798 mouse lymphosarcoma, tumor differences in the properties of the hormone receptors in the sensitive and resistant cells have been noted (10, 15). However, in this tumor line (11) and at least in some instances in human leukemias (4), cell resistance appears also to occur at steps beyond those associated with glucocorticoid binding. A novel mechanism for this resistance to hormone-induced cell killing has been suggested by observed structural differences between the sensitive and resistant lines. It appears that the emergence of resistance occurs via the selection of cells that have hardier membranes, those that are better able to survive the intracellular changes brought about by glucocorticoids (11). This mechanism has received support from observations of differences in nuclear fragility between sensitive and resistant lines of lymphosarcoma cells that are detectable in the absence of hormones.

The present study was undertaken to examine the possibility that changes in individual cellular proteins were involved in the emergence of the hormone-resistant state. Utilizing 2-dimensional gel electrophoretic separations [modified from the data of O’Farrell (13)], we compared the rates of synthesis of about 1000 soluble proteins. While the synthesis of most proteins was similar in the sensitive and resistant tumor cells, some distinct changes were noted. One of the most prominent of these was the appearance of a new protein associated with the highly resistant state. There were also increases in 4 other proteins and decreases in 2. The results suggest that resistance to glucocorticoid killing in P1798 lymphosarcoma cells could be related to relative rates of synthesis of a few cellular proteins.

MATERIALS AND METHODS

Tumor Cells. At 3-week intervals, the sensitive and resistant lines of the P1798 tumor were implanted into male BALB/c mice (Jackson Laboratory, Bar Harbor, Maine) 4 to 6 weeks old. Where specified, tumors were implanted in mice that had been adrenalectomized less than 1 week prior to passing. Tumors were removed from the animal under aseptic conditions, and cells were dispersed into a sterile 0.9% NaCl solution with a spatula. Cells were then passaged into the flank of the mice by a syringe with a 22-gauge needle. The tumor lines were routinely tested for in vivo hormone sensitivity or resistance by the procedure of Kaiser et al. (8). Tumors in mice treated for 3 days with hydrocortisone acetate (75 mg/kg/day; Upjohn Co., Kalamazoo, Mich.) regressed from 5% (highly resistant) to 75% (highly sensitive) of the control values. Following cervical dislocation, the 17- to 20-day-old tumors were removed from the mice and minced in tissue culture medium RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.). Cells were then washed in RPMI 1640 minus methionine (Grand Island Biological Co.), filtered, and resuspended (in RPMI 1640 minus methionine) at a concentration of 5 x 10^6 cells/ml. Throughout this procedure, the medium was maintained in equilibrium with 95% O_2-5% CO_2.

Aliquots of 100 µCi [35S]methionine (500 to 1200 Ci/mmol; American Radiolabeled Chemicals, Arlington Heights, Ill.) are evaporated in small incubation vials. The cell suspension (1 ml) was preincubated in a 10-ml Erlenmeyer flask at 37° under 95% O_2-5% CO_2 in a Dubnoff metabolic shaking incubator (100 cycles/min) for 10 min. Aliquots (100 µl) were then added to the small vials containing [35S]methionine and incubated as described for 1 hr. Cells were never incubated in the presence of hormone. Cells were washed with 1 ml of complete RPMI 1640, collected by centrifugation (20 sec, 1000 x g, refrigerated), and dissolved in 100 or 200 µl lysis buffer (13). Radioactivity was determined (7), and samples were frozen at -79°.

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Blood and Fibrous Tissue. Whole-blood samples are taken from mice bearing both corticosteroid-sensitive and -resistant tumors. Immediately following cervical dislocation, the sample of blood (0.2 ml) is taken by cardiac puncture with a 1-ml syringe (22-gauge needle) and diluted into 50 ml complete RPMI 1640. These samples are centrifuged for 2 min in a clinical centrifuge and then washed twice in 25 ml RPMI 1640, washed once in RPMI-1640 minus methionine, and filtered. Hematocrits are taken, and the cells were resuspended (in RPMI 1640 minus methionine) at the same packed cell volume as the tumor cells (5 × 10^6 tumor cells/ml, =2% packed cell volume). The cells from blood are then labeled and incubated as described above for the tumor cells.

That portion of the tumor that does not pass through the nylon mesh during the tumor cell preparation is considered to be fibrous tissue associated with the cells. This fibrous material from both corticosteroid-sensitive and -resistant tumors is washed twice in complete RPMI 1640 and once in RPMI 1640 minus methionine. Two or 3 small pieces of this material, of weight equal to that of 100 µl of tumor cells (=0.5 to 1.0 mg), is incubated in RPMI 1640 minus methionine and labeled as described above for the tumor cells.

Two-dimensional Gels. IEF and NEPHGE were carried out as described (13, 14). Duplicate samples dissolved in lysis buffer [9.5 m urea (ultrapure; Schwarz/Mann Orangeburg, N. Y.), 2% (w/v) Nonidet P-40 (Particle Data Labs, Elmhurst, Ill.), 2% amphotolines (1.6% of pH range 5 to 7 and 0.4% of pH range 3.5 to 10; LKB Instruments), and 5% β-mercaptoethanol (Sigma Chemical Co., St. Louis, Mo.)] and containing 500,000 cpm in less than 10 µg of protein were applied to both IEF and NEPHGE gels. After prerunning (0.25 hr 200 V, 0.5 hr 300 V, 0.5 hr 400 V), IEF gels were focused for 15 hr at 400 V followed by an additional h at 800 V. NEPHGE gels were run for 4 hr at 400 V. Immediately following completion of the run, the power was turned off, and the gels were gently extruded into test tubes containing 5 ml of SDS sample buffer [10% (w/v) glycerol, 5% (v/v) 2-mercaptoethanol, 2.3% (w/v) SDS (Bio-Rad, Richmond, Calif., and 0.0625 M Tris-HCl, pH 6.8 (13)]. Gels were equilibrated for 2 hr with 2 × 5 ml SDS sample buffer at room temperature and then either run immediately or quick frozen by placing the test tubes in an ethanol bath at −80°C. The second dimension was run using a 4.5% stacking gel and 10 to 16% exponential acrylamide gradient (5 ml in the front chamber). After the first-dimension gel was sealed to the slab gel, electrophoresis was for approximately 3.5 hr at 20 ma/gel constant current until the bromophenol blue tracking dye reached the bottom of the gel. Gels were washed in 7.5% acetic acid and dried under heat and vacuum. Some gels, containing molecular weight standards were stained in 0.1 Coomassie Blue R250 (Bio-Rad) in 30% methanol-7.5% acetic acid and destained in 10% methanol-7.5% acetic acid. Dried gels were exposed to Kodak X-omat R X-ray film for the indicated times and then processed in an X-omat automatic processor.

Qualitative and Quantitative Analysis of Protein Peaks on Gels. There is some variability in relative densities of proteins in individual gels that is a characteristic of 2-dimensional electrophoretic separations. These are large enough to render quantitative determinations from a single gel unreliable. Therefore, to find the most consistent differences among groups, our approach has been to run duplicate samples and to compare the data from repeated similar experiments. Criteria for consistency of observed changes were as follows. If the protein density increased or decreased by visual examination in at least 3 of the 4 experiments and in at least 10 of the 20 gel comparisons, the protein difference was considered consistent. (Protein Spot 41 was an exception; it appeared in all 4 of the experiments and 6 comparisons of the 20. It was included, however, because it was located on the basic edge of the IEF gel, it occasionally appeared on the acidic end of the NEPHGE gels, and frequently it did not appear on either gel. Had our procedures involved a consistent isolation of this protein, it probably would have met the original criteria for a consistent difference.)

Cursory examination of autoradiograms from many gels from both sensitive and resistant cells reveals a general uniformity in patterns of the protein spots with little apparent variation in the relative densities of these spots. However, there were a few proteins that exhibited clear-cut changes. Attempts were made to roughly quantitate these rather obvious changes by comparing the relative densities of the variant spots with neighboring invariant ones. In addition, 3 control spots that did not change with the state of resistance were also compared to variant spots. The invariant comparison spots were chosen on the basis of 2 criteria: (a) because of variations in background density (and occasionally variations in the resolution quality across a gel), the invariant spot was selected to have approximately the same density as the variant spot in its most dense condition. Densities of the variant spots were then rated on a scale of 0 to 5 as a measure of their relative density to the neighboring invariant ones. Spots of equal density were assigned the number 3, those with increased densities were assigned the number 4 or 5, and those with lesser densities were either 0, 1, or 2, signifying no visible spot, 1 signifying roughly one-third the density and 2 signifying two-thirds the density. Relative densities were estimated in a blind study by 2 independent observers. The data represent an average of these 2 estimates; in most cases, both observers gave identical estimates for the relative densities of the individual protein spots. Although duplicate samples from the same experiment yielded highly reproducible spot densities, variations seen between experiments appear, in retrospect, to be the result of a steep spot density versus hormone sensitivity curve (see Chart 2). While it should be appreciated that such a system can give only rough semiquantitative results, this system nevertheless was deemed sufficient to deal with the relatively large changes in the density that were seen.

We gained some assurance of the applicability of using visual estimates to measure total spot density by comparing the relative spot densities obtained by varying the length of exposure time of the X-ray film to the same gel. When film exposures are corrected for isotope decay and densities of the spots are arranged according to the same 0 to 5 scale, the result is an approximately linear relationship between total spot density and log of the exposure times. A progression from one integer to the next on the scale (say from 2 to 3) represents an approximate doubling of the total apparent density of the spot.

RESULTS

These studies were aimed at detecting changes in the more rapidly synthesized soluble cellular proteins. They utilize 2 different separatory systems, IEF gels for proteins with pI’s of ≈4.5 to 7.5 and NEPHGE gels for proteins with pI’s greater than ≈7.5. Multiple aliquots from single samples of incubated cells were run on both gel systems. As applied to P1798 lymphosarcoma cells, these methods yield ≈450 proteins on IEF gels and ≈425 proteins on NEPHGE gels. There is very little overlap in the proteins separated on the 2 systems.

Data from IEF gels show that the majority of cellular proteins synthesized in 1 hr by corticosteroid-sensitive lymphosarcoma cells appear to be the same as those synthesized by corticosteroid-resistant cells. Representative gels are shown in Fig. 1. (The peptide numbers that identify individual peaks on the figures are arbitrarily assigned.) Among the more acidic proteins, there are 4 with densities that are consistently increased in the resistant line as compared to the sensitive line (Table 1). These are Peaks 19 (M.W. ≈85,000, pl 5.9), 41 (M.W. ≈75,000, pl 8), 2, and 3 (M.W. ≈70,000, pl 6.9). The last 2, barely distinguishable as separate peaks, are uncommon proteins in these gels in that they appear to be more dispersed.
than most proteins in both pl and molecular weight. Among those proteins with a pl greater than 7, there are 2 with densities that decrease in the resistant line, Protein Spots 101 (M.W. = 17,000) and 149 (M.W. = 60,000) (Fig. 2). One of the largest differences seen between sensitive and resistant tumor cells is the appearance of the Protein Spot 110 (M.W. = 36,000), which usually appears as a dense spot in resistant cells and has never been observed as more than a faint spot in sensitive cells.

Control experiments were done to determine if variations in these 7 proteins were possibly the result of a variable contamination of the tumor cell preparations by other types of cells. Two likely candidates are constituents of blood that might result from an unequal blood supply or fibrous tissue from fibroblasts surrounding the tumors. Accordingly, samples of washed blood cells and of fibrous tissue associated with the tumor cells, from both corticosteroid-sensitive and -resistant tumor-bearing animals, were labeled and incubated at the same time as tumor cells ("Materials and Methods"). These possible contaminants were incubated at an equal volume (washed blood cells) or an equal weight (fibrous tissue) to the tumor cells. In both cases, the cell samples were dissolved in 100 μl of lysis buffer, and the same size aliquot was applied to the gels as was applied for the sensitive and resistant tumor cells. In these experiments, tumor cells incorporated = 600,000 cpm [35S]methionine per μl lysis buffer; washed blood cells and fibrous tissue incorporated = 25,000 cpm/μl. Washed blood cells have only 2 major proteins that incorporate methionine; both are relatively small and are pigmented (both have a molecular weight of ~14,000 and are separated on NPHGE gels by a difference in pl). It is probable that these are the α and β subunits of hemoglobin; these subunits are similar in molecular weight, the α-subunit being more acidic than the β-subunit. Fibrous tissue incorporates very little [35S]methionine into any of the proteins. However, if the gels are exposed for very long times or if very large samples (with the same amount of radioactivity as tumor cells) are put on gels, the labeled proteins found in the fibrous tissue sample are shown to be the result of tumor cells in the sample. Moreover, neither blood nor fibrous tissue has major protein spots in any of the 7 locations of protein differences seen in sensitive or resistant tumor cells. Thus, even if blood components or fibrous tissue had represented major contaminants of the tumor sample, they could not account for the differences seen between corticosteroid-sensitive and -resistant lymphosarcoma cells.

Since these experiments were not routinely done using adrenalectomized animals, the differences between the sensitive and resistant state in proteins could conceivably be due to the induction (or suppression) of individual proteins in the sensitive tumor by the glucocorticoids present in the host animal. However, we find that all 7 protein differences are present to approximately the same degree when the sensitive tumors are grown in either adrenalectomized or normal mice. This indicates that differences between proteins of the hormone-sensitive and -resistant cells are probably not the result of induction or suppression of the proteins by endogenous glucocorticoids.

We have attempted to express the degree of sensitivity of the tumor to glucocorticoid killing in vivo as a function of the relative densities of the 7 protein spots. In such experiments, sensitivity to hormone is measured in vivo by tumor-bearing mice given injections of pharmacological doses of hydrocortisone acetate for 3 consecutive days. After 36 hr, the tumors are removed and weight recorded. The decrease in tumor weight in the sensitive line is the result of 2 factors, a slowing of the rate of growth and tumor regression (presumably due to cell death). The results of a typical experiment (Chart 1) show, in this particular case, a 60% decrease in tumor weight in the sensitive line following treatment with hormone but only a 5% decrease in the resistant line. Alterations in the degree of sensitivity measured in this manner are related to changes in the relative density of the 7 protein spots (Chart 2).

### Table 1
Protein differences between glucocorticoid-sensitive and -resistant P1798 lymphosarcoma cells

<table>
<thead>
<tr>
<th>Protein spots</th>
<th>M.W.</th>
<th>pl</th>
<th>Magnitude of change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in the resistant state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>101</td>
<td>17,000</td>
<td>&gt;7.6</td>
<td>1.3</td>
</tr>
<tr>
<td>149</td>
<td>60,000</td>
<td>&gt;7.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Increase in the resistant state</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 and 3</td>
<td>70,000</td>
<td>6.9</td>
<td>1.2</td>
</tr>
<tr>
<td>19</td>
<td>85,000</td>
<td>5.9</td>
<td>1.2</td>
</tr>
<tr>
<td>41</td>
<td>75,000</td>
<td>8</td>
<td>1.2</td>
</tr>
<tr>
<td>110</td>
<td>36,000</td>
<td>&gt;7.6</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Chart 1. Glucocorticoid sensitivity of P1798 corticosteroid-sensitive and -resistant lines in vivo. Mice bearing 8- to 13-day-old tumors received s.c. injections (0.1 ml) of hydrocortisone acetate (F), 75 mg/kg/day in 0.9% NaCl solution, or 0.9% NaCl solution alone for 3 consecutive days. Thirty-six hr after the last injection, the mice were sacrificed, and the tumors were removed and weighed. Open bars, weight of tumors from mice given injections of 0.9% NaCl solution; hatched bars, tumor weights from mice given injections of hydrocortisone acetate. Data presented are mean for 4 mice. Bars, S.E. in the sensitive line, there is a 60% decrease in tumor weights between control and hormone treated, and this difference is significant at p < 0.002. In the resistant line, there is a 5% decrease in tumor weight with hormone treatment, and it is not significantly different from the control.
Proteins Associated with Glucocorticoid Resistance

Over a period of time, the relative sensitivity or resistance of our 2 tumor lines varies somewhat. When not periodically (once every 3 to 4 months) selected for highly resistant cells (by treatment in vivo with pharmacological doses of glucocorticoids), the resistant line gradually tends to become more sensitive. This trend has been observed several times over 3 years. When the degree of resistance (determined as in Chart 1) is compared to the relative density of the 7 protein spots (Spots 2, 3, 19, 41, 101, 110, and 149), highly reproducible changes are observed (Chart 2). The changes are especially striking when the cells become highly resistant (when the decrease in tumor weight following administration in vivo of hydrocortisone acetate (F) (as described in the legend to Chart 1). This chart represents accumulated data from 10 experiments (each experiment usually contained duplicate gels run on each of the 2 sensitive and the 2 resistant samples). Points, data from a single gel. In the majority of cases, relative densities at exactly the same position on the abscissa represent data from one experiment. Tumors of all gradations in the degree of resistance (from fully sensitive to fully resistant) were included in this comparison. It should be noted that for this figure the relative density is similar to "percentage of control" and control spots were different for each variant spot. For example, a relative density of 3 for Protein Spot 110 is much more dense than it is for Protein Spots 2 and 3. For approximate comparisons of the magnitude of the changes in these proteins, see Table 1.

One possible explanation for the apparent curvilinear relationship between spot density and degree of resistance is that among the sensitive tumors the degree of sensitivity might not be solely a function of the properties of the individual cell. It has been suggested that sensitivity of the tumor to the hormone when the animal is treated in vivo could be influenced by the size of the tumor (5). However, such a tumor size-regression relationship appears to be ruled out by data presented in Chart 3. Since a number of the most resistant tumors fall within the size range of the most sensitive ones, the degree of sensitivity among the sensitive tumors is probably not altered significantly by the size of the tumor when hormone is administered.

DISCUSSION

Our earlier work on P1798 lymphosarcoma cells indicates that hormone-induced increases in nuclear fragility, presumed to be the forerunner of the lethal actions, are receptor-mediated effects since the presence of cortesolone, a competitive antagonist of glucocorticoid binding, protects the cells from the onset of deleterious effects. Those studies also suggested that resistance may not be solely attributed to a lack of hormone receptors, since one can observe effects on nuclear fragility of the same magnitude in both the sensitive and resistant cells. The presence of a hormone effect in the resistant population a priori would indicate the presence of receptors. Further, one could differentiate between sensitive and resistance cells in the absence of hormone on the basis of differences in cell survival.

Chart 2. Relative changes in radioactivity in individual proteins as function of the degree of glucocorticoid sensitivity of the tumors in vivo. Cells are labeled for 1 hr with $[^{35}S]$methionine, and 2-dimensional gel electrophoresis is carried out as described in "Materials and Methods." Ordinate, relative densities (on the autoradiogram) of the 7 consistently variant protein spots and 3 control invariant spots. These were related on a scale of 0 to 5 by visual comparison in a "blind" study (see "Materials and Methods") to the density of a neighboring protein spot that does not vary with the state of resistance. Zero indicates no visible spot. Abscissa, percentage of decrease in tumor weight following administration in vivo of hydrocortisone acetate (F) (as described in the legend to Chart 1). This chart represents accumulated data from 10 experiments (each experiment usually contained duplicate gels run on each of the 2 sensitive and the 2 resistant samples); points, data from a single gel. In the majority of cases, relative densities at exactly the same position on the abscissa represent data from one experiment. Tumors of all gradations in the degree of resistance (from fully sensitive to fully resistant) were included in this comparison. It should be noted that for this figure the relative density is similar to "percentage of control" and control spots were different for each variant spot. For example, a relative density of 3 for Protein Spot 110 is much more dense than it is for Protein Spots 2 and 3. For approximate comparisons of the magnitude of the changes in these proteins, see Table 1.
that correspond with the observed differences in nuclear fragility (12). The latter property is perceived to be related to changes in the nuclear membranes. Other investigators have found differences in the negative charges on cytoplasmic membranes of the corticosteroid-sensitive and -resistant lymphosarcoma cells (1, 2). Our working hypothesis, formulated from such considerations, is that resistance emerges via the selection of cells with harder membranes that are hence better able to withstand the intracellular changes initiated by glucocorticoids (11, 12).

The present study was undertaken to examine the possibility that differences between the cellular proteins of corticosteroid-sensitive and -resistant cells might be involved in the emergence of the resistant state. The cells were labeled with $^{35}$S-methionine for 1 hr. Our earlier studies demonstrated that, during this relatively short time of incubation, neither resistant nor sensitive cells show any significant deterioration (11).

On the whole, the proteins labeled in the sensitive and resistant lymphosarcoma cells are similar. Indeed, one would expect such homology between these 2 sets of proteins, since the resistant line was originally derived from the sensitive line. However, findings (a) that in both cell lines proteins are labeled at approximately the same rates and (b) that the overwhelming majority of proteins are about the same density in sensitive and resistant lines both provide evidence against changes in the amino acid pools.

The rather crude measurement of relative densities of these 7 variant proteins and 3 control invariant proteins was done visually by comparison with neighboring invariant spots on the same gel. The relative densities represent the average of estimates made by 2 independent observers in a blind study where neither observer knew which of the 10 protein spots were believed to change. While this technique cannot be relied upon for fine discrimination, changes in the 7 proteins were sufficiently large to be detected. It appears as if these relative densities are related to the degree of sensitivity of the tumor to glucocorticoid killing in vivo. When the data are plotted as a function of the degree of glucocorticoid sensitivity, all of the resulting curves seem to be diphasic. For example, when the tumor is in a highly resistant state, small changes in the degree of resistance coincide with major changes in the densities of the protein spots. A possible explanation for these changes in the slope of the curve is that its shape reflects a nonlinearity in the technique for quantitation. It is possible that for 3 of the spots (Protein Spots 2, 3, and 4) the minimum level of detection might have been reached. However, even longer exposures of the film do not reveal significant spots in the sensitive state. This suggested explanation seems even less likely for those proteins that decrease in the resistant state (Protein Spots 101 and 149). Here the densities fall on the linear portion of the film response curve, definitely not near saturation capacity of the X-ray film. This was confirmed by comparing a set of standardization wedges (13) to the maximum densities of the spots by densitometry. Also, visual comparisons of spot densities versus exposure times suggest an appropriate linearity between the relative changes in exposure to radioactive disintegrations and scoring by the method used (described in "Materials and Methods"). From these considerations, it seems improbable that the biphasic curve is the result of nonlinearity. Several laboratories are developing computerized analysis for measuring densities of chromatographic spots (3, 6). There seems little doubt that when such techniques are widely available they will add greatly to our understanding of the subtle differences between protein peaks on 2-dimensional gels.

The phenomena of glucocorticoid-sensitive cells becoming resistant is also observed in normal cells. Glucocorticoid-sensitive thymus cells mature and eventually become immunologically committed and resistant. Using normal rat thymus cells as another model system for resistance, we find consistent differences between "sensitive" and "resistant" thymus cells.

Resistant rat thymus cells are selected by treating rats with dexamethasone. When the proteins from these cells (labeled with [35S]methionine in vitro) were analyzed by 2-dimensional gel electrophoresis, 18 consistent differences were observed. An intriguing observation is that one of the major proteins present in the resistant rat thymus cells, but not in the sensitive cells, apparently is in the same position on the gels as the largest protein change seen in resistant lymphosarcoma cells [Protein Spot 110 (Fig. 2)]. In fact, when proteins from resistant thymus cells and proteins from the resistant tumor cells are run on the same gel, these 2 proteins coelectrophorese. Experiments currently in progress are designed to determine the intracellular location of the protein differences.

ACKNOWLEDGMENTS

We are grateful to Mary Wilkey for her help in typing the manuscript and to Dr. Fred Rosen, Roswell Park Memorial Institute, Buffalo, N. Y., for providing the initial lymphosarcomas for transplantation in these studies. We are especially grateful to both Ingrid Wood and Peter Gottlieb for the time they spent visually estimating the densities of over 500 protein spots.

REFERENCES

Fig. 1. EF of P1798 corticosteroid-sensitive and -resistant lymphosarcoma cells: proteins. Cells were labeled with [35S]methionine, and 2-dimensional gel electrophoresis was carried out as described in "Materials and Methods." The dried gel was exposed for 6 days. In these 2-dimensional IEF gels, the first dimension (IEF) was run from left to right (basic end of pH gradient on the left and acidic on the right); the second dimension (SDS-polyacrylamide gel electrophoresis) was run from top to bottom. In all figures of 2-dimensional gels, the numbers (which were arbitrarily assigned) refer to proteins described in the text. Arrows upward, a consistent increase in density of the protein spot for that experimental condition; arrows downward, a consistent decrease.
Fig. 2. Nonequilibrium pH gradient electrophoresis gels of P1798 corticosteroid-sensitive and -resistant lymphosarcoma cellular proteins. Cells were incubated with [35S]methionine and 2-dimensional gel electrophoresis was carried out as described in "Materials and Methods." The dried gel was exposed for 6 to 7 days. In these 2-dimensional NEPHGE gels, the first dimension was run from right to left (basic and of pH gradient was on the left and acidic end of the right); the second dimension was run from top to bottom.
Proteins Associated with Emergence of the Resistance to Lethal Glucocorticoid Actions in P1798 Mouse Lymphosarcoma Cells

Mary L. Nicholson, Bruce P. Voris and Donald A. Young