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Protein Abnormalities in Macrophages Bearing Asbestos

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Computerized techniques for the evaluation of O'Farrell two-dimensional electrophoretic gels have been applied to proteins derived from asbestos bearing macrophages. Preliminary results indicate definite changes in the protein content of cells depending on fiber phagocytosis.

Introduction

The protein components of macrophages treated with amosite asbestos were examined by two-dimensional gel electrophoresis as described by O'Farrell (*1*). The resulting complex patterns were analyzed by using computerized techniques (*2*).

Because of its phagocytic activity, the macrophage is an essential part of the pulmonary defense inhaled particles. In the presence of asbestos particles macrophages have been reported to undergo a series of morphological (*3*) and biochemical (*4*) changes.

The results presented here indicate that amosite asbestos produces numerous changes in the protein composition of macrophages. The major emphasis will be focused on two spots in the autoradiograph which lie side by side in a region defined as an isoelectric point of 5.4 to 5.2 on isoelectric focusing gels and a molecular weight near 45,000 as defined by mobility in SDS-polyacrylamide gels. Further studies are in progress in an attempt to more fully characterize these spots.

The computer-aided gel analysis system used in this study has been previously described (*2*). This system consists of a general purpose image processing system with a subset of programs, called the FLICKER system, designed for two-dimensional analysis.

$P388D_1$ macrophage-like cells were grown in tissue culture. Polypeptide differences were observed in whole cell lysates by two-dimensional electrophoretic techniques as described by O'Farrell (*1*).

Sample Preparation

Asbestos-Challenged Macrophages in Tissue Culture

The cells analyzed are the same as those reported previously in cytotoxicity studies (*5*). $P388D_1$ macrophage-like cells were grown in monolayers in tissue culture. Wherever the term "macrophages" is used in an unqualified sense in this paper, it refers to $P388D_1$ macrophagelike cells in tissue culture. After 24 hr, selected flasks are challenged by asbestos fibers in various concentrations. For this experiment, 50 $\mu g/cm^3$ UICC (International Union against Cancer — Standard amosite B preparation) standard amosite was used. The cells are labeled with a mixture of ^{14}C-labeled amino acids (at 5 $\mu Ci/ml$) 24 hr before harvesting.

During the experiment, selected zones of the monolayer are manually counted at three consecutive 24-hr intervals for the controls and the challenged cells (24, 48, 72 hr). The relative number of surviving cells is used as the measure of cytotoxicity. Because the cultures employed here were also being used for the bioassay of cytotoxicity, some specific details of the protocol bear on the quantitative results of two-dimensional gel analysis. In particular, a

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media change at a time approximately 48 hr after the initial (baseline) count is required for continued cell viability. This change of media undoubtedly reduces total cell number and, since practically all cells bear fibers, there is a concomitant reduction in total fiber number within a given flask.

Preparation of Two-Dimensional Electrophoretic Gels from TC Samples

Harvested cells were scraped from the flasks and centrifuged at 8000XG, the supernatant was discarded. The pelleted cells were then lysed and denatured by addition of a solution containing 2% SDS, 5% mercapoethanol, 20% glycerol, 2% 3/10 Biolytes (Bio-Rad) and 2% NP-40, followed by heating for 5 min at 95°C.

Two-dimensional electrophoresis was performed as described by O'Farrell, by use of 3/10 Biolyte (Bio-Rad) in the first dimension and a 10% acrylamide uniform gel in the second dimension. Isoelectric focusing was performed for 20 hr at 500 VDC. Slab gels were run at 20 mA/gel.

Autoradiography was performed by first soaking the gels in a preserving solution (methanol: H_2O:glycerol, 70:27:3 v/v) for 5 min. These gels were then dried on #3 MM filter paper (Whitmann) at 50°C under a vacuum for 2 hr. The dried gels were then placed in x-ray film cassette holders and exposed at −70°C for appropriate intervals. Films were developed at 25°C for 5 min in Kodak KLK developer followed by fixing (5 min) in a Kodak X-omatic fixer. Gel polypeptide patterns are displayed with the acid pH range on the right and the low molecular weight polypeptides at the bottom as seen in Figure 1.

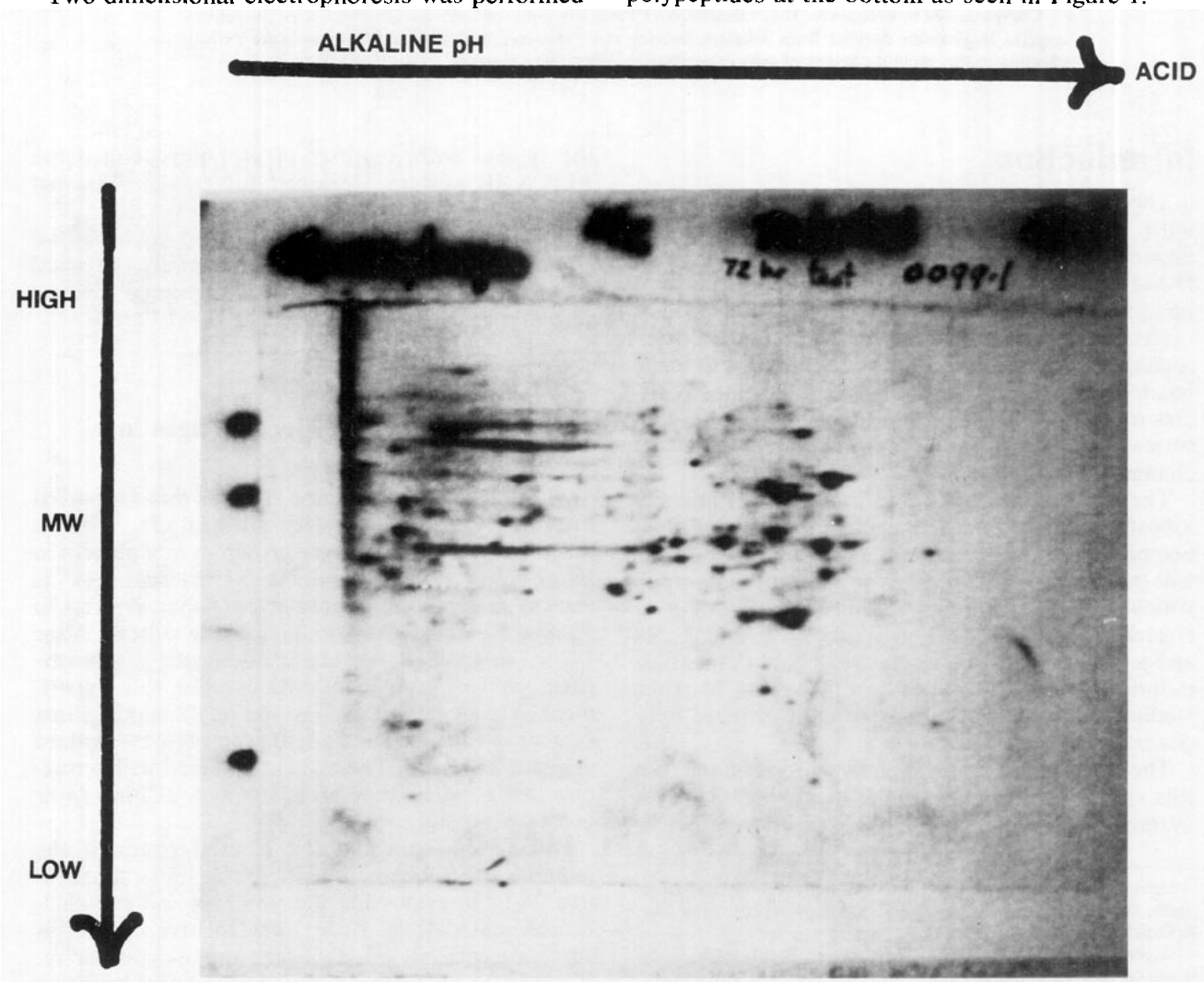


FIGURE 1. Orientation of pH/MW two-dimensional electrophoretic gel gradients. The pH varies from alkaline (left) to acid (right), while molecular weight varies from high (top) to low (bottom). The gel is first run on a isoelectric pH gradient as a one-dimensional gel. This is then treated with SDS and run as a two-dimensional gel to gel the molecular weight gradient.

Gel Analysis Problems

Because of the number of spots and various local translational and rotational distortions in the gels, it is often difficult to determine differences between gels at the individual spot level. In addition, spots may vary in density, size, shape, and — as a consequence of distortion — local relative position.

In order to compare corresponding spots in the two gels, overlaying of the autoradiographs is necessary. However, due to gel distortions, spot clusters may only be compared over (often small) local regions. Manual overlay may be useful for detecting obvious differences. Subtle differences in a heavily populated spot field are difficult to detect manually.

Computer-Assisted Analysis of Gel Images

There are several ways that the computer analysis of gel images may be used to aid in spot analysis: flicker comparison of gels to detect spot differences, spot densitometry, automatic spot segmentation and comparison, and spot data management.

FIGURE 2. Light box and TV camera. The gel is placed on the light box above the NBS standard ND step wedge and a 512 × 512 point 256-gray level image acquired by the RTPP for later analysis.

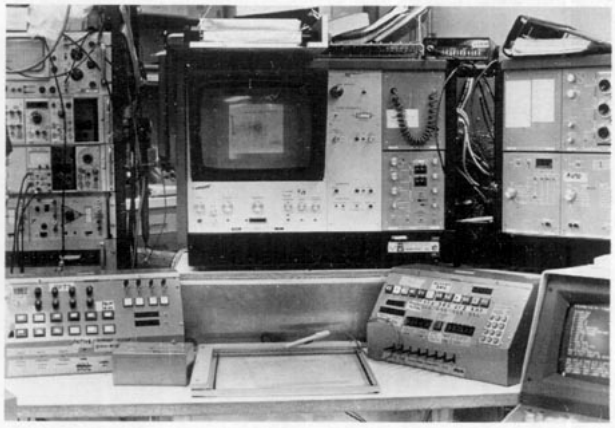
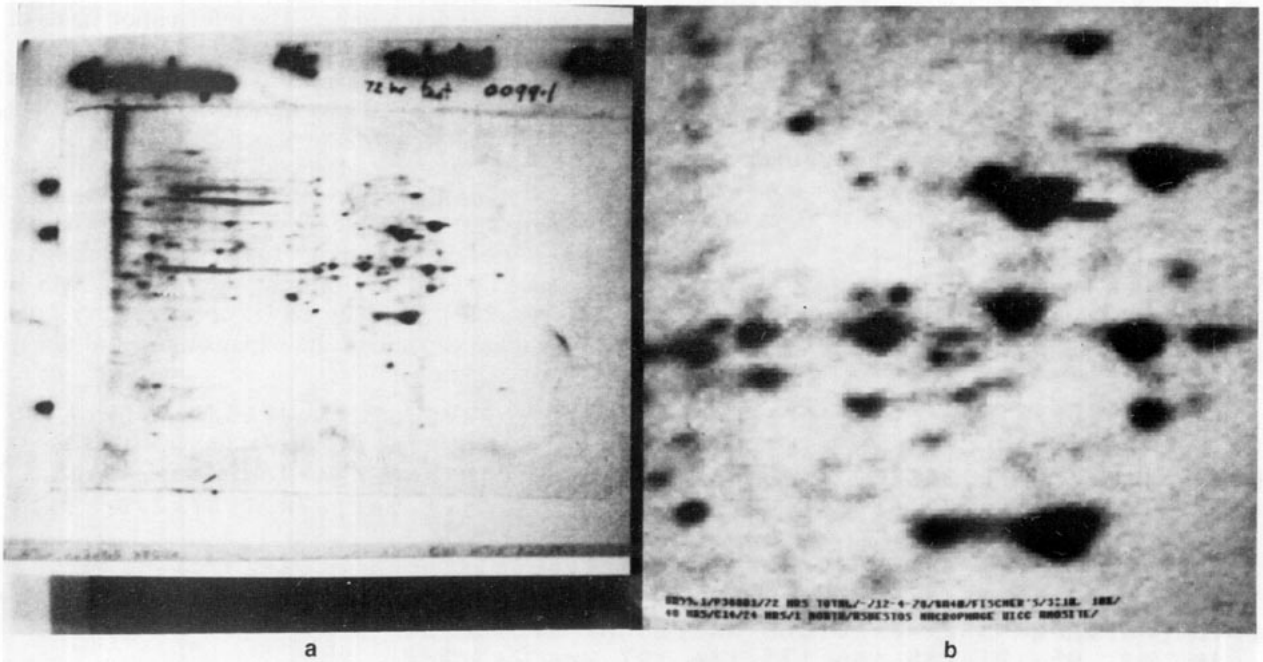


FIGURE 3. Operator's console of the RTPP. The gel images may be displayed on the TV and various analysis operations invoked from the console keys.



a b

FIGURE 4. Amosite 72 hr gel: (*a*) 0099.1 at 1X, (*b*) 99.1 at 4X magnification.

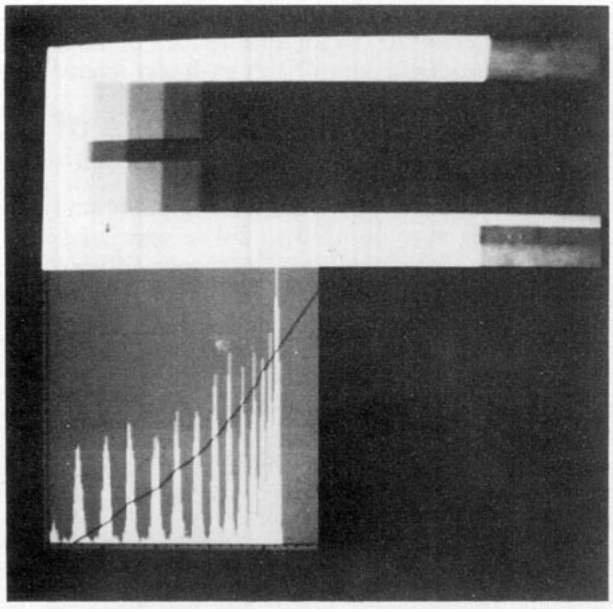


FIGURE 5. NBS step wedge (top) and its piecewise linear calibration (bottom).

Gel Scanning Procedure

Initially, the autoradiograph is placed on a light box where it is then scanned by the computer system. Figure 2 shows a Picker light box used to backlight the autoradiographs of the two-dimensional gels. Notice the neutral density (ND) wedge at the bottom of the light box. This is scanned by the Vidicon TV camera at the same time as the gel in order to calibrate density measurements to the NBS ND step wedge. Images are acquired by using the BMON2 image processing software system (*6, 7*) running on the Real Time Picture Processor (RTPP) system (*8-10*).

Figure 3 shows the operator's console of the image processing system which a researcher uses to analyze images previously acquired.

Computer Facilities

There are four major facilities provided the user in this system in addition to image acquisition.

The system permits the flicker comparison of gel images at various electronic magnifications (as is illustrated in Figs. 4*a* and 4*b*) at variable flicker rates. Spot differences may be recorded using a spark pen and tablet to point to spots.

Densitometry may be performed on manually selected spots by the operator drawing a boundary around the spot to be measured. Figure 5 shows the NBS-calibrated ND step wedge and its resultant calibration curve used to calibrate density samples relative to the wedge.

The automatic spot segmentation and comparison of spots between gels comprising hundreds of spots (including the density of each spot) are used to make thorough analyses of the gels.

A data management system is used to facilitate the analysis of intra- and intergel differences between sets of spots in multiple gels. These data structures include: (a) gel identification file, processing history, and characteristics (ND wedge calibration, etc.); (b) canonical map (C-maps) file for defining subsets of C-maps of spots (to be discussed); (c) gel spot density data files (GSDF) of manually measured spot densities; (d) gel segmentation files (GSF) used in automatic gel comparisons. The latter are used to compute transitive closures of spot differences and comparisons between all gels with each other for subsets of gels.

Gels are identified by an accession number. A global file exists where all gel information accessible to the system is kept, one entry/gel image. All gels, once entered into the system may then be referenced by their accession number. The information fields of a record for a typical example are shown in Figure 6.

Automatic or semiautomatic specification of pairs of gels to be analyzed may be selected from the data base based on experiment specific criteria.

The large number of spots, their locally variant positions and density make a powerful data management system (DMS) essential if quantitative intergel spot differences are to be tracked through a sequence of gels. The present state of the DMS is severely constrained by CPU, core memory, and disk storage capacities. Its expansion to full utility

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ACC#/PATIENT/BIRTHDATE/RACE&SEX/EXP DATE/EXP#/CULTURE REAG/AMP,GEL/
INTRVL BEFR LBLNG/LBLNG ISOTOPE/DURTN LABEL/DURTN OF EXPRS/STUDY/
FILE#/TAPE#/OPT. BACKUP TAPE /CAMERA,LENS,DISTANCE/EXPRMNTR*
ND:.05,.20,.35,.50,.66,.80,.95,1.10,1.25,1.41,1.56,1.72,1.87,2.02,2.17

0099.1/P388D1/96 HRS TOTAL/-/12-4-78/#A40/FISCHERS MEDIA/3:10, 10%/
72 HRS/C14/24 HRS/1 MONTH/ASBESTOS MACROPHAGE UICC AMOSITE B/
L00061/R602/--NONE--/VIDICON-MAN,28MM,F8,69CM/LIPKIN*
  38  63  95 121 139 156 173 186 197 206 213 222
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FIGURE 6.

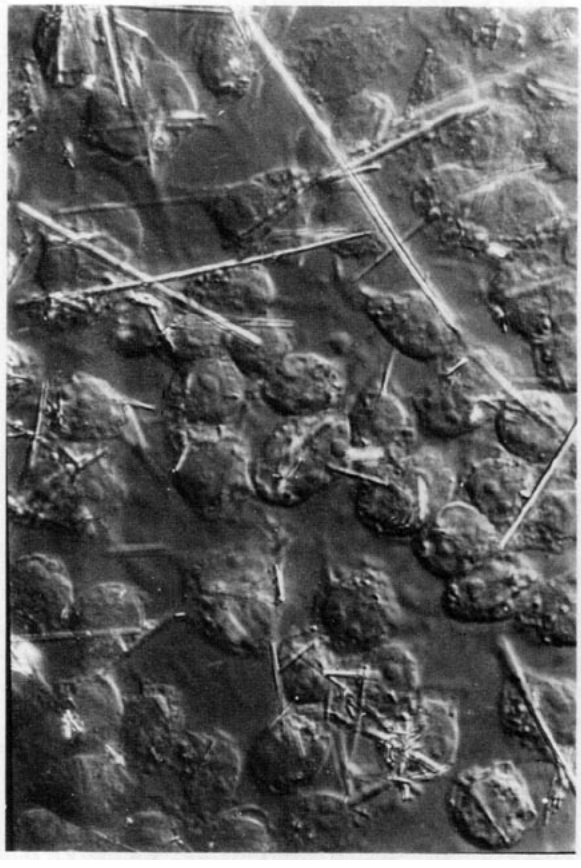


FIGURE 7. Photomicrograph of the macrophage with amosite after 24 hr.

must await the anticipated installation of a dedicated medium size computer facility.

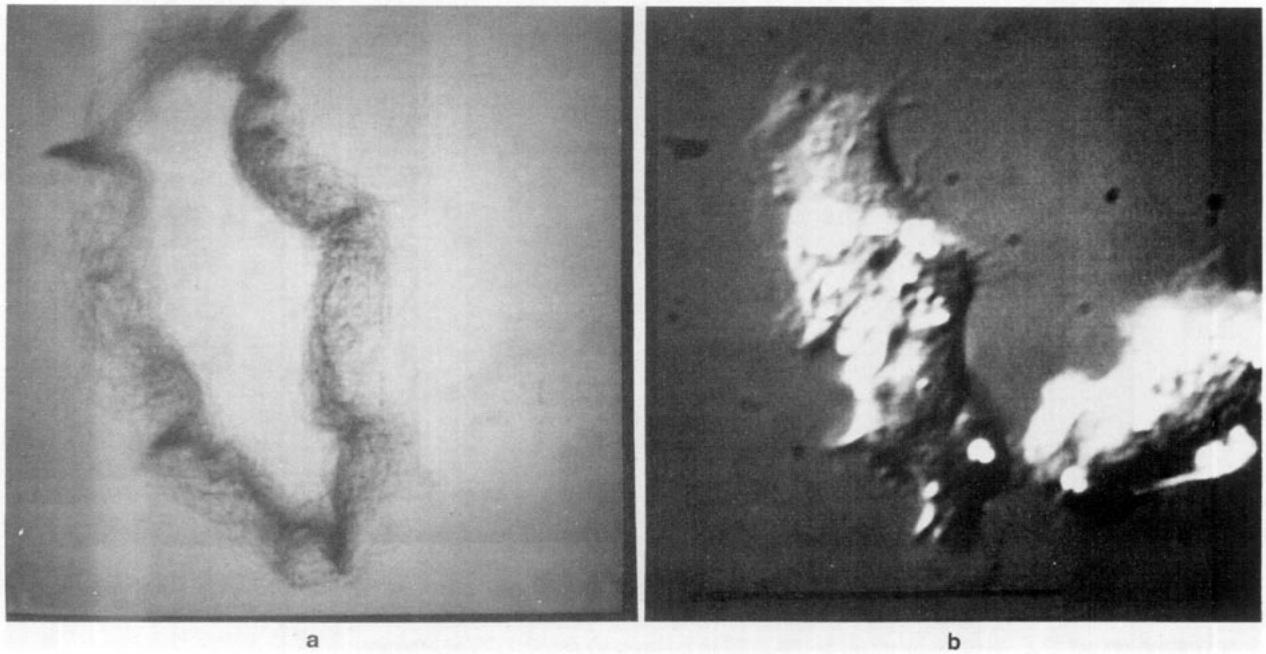
The Biological System

Figure 7 shows a TC sample 24 hr after introduction of amosite. The differential interference photomicrograph was taken at 400×.

Several cytoskeletal morphologic changes are apparent when seen under differential interference microscopy. The cell exposed to amosite is (a) flatter and more adherent to glass, (b) exhibits less translational motion, and (c) has possibly less nontranslational motion (observed over time) although not completely absent. The boundary trace transform (BTT) (*11, 12*) has been used as a measure of these latter two phenomena. Figure 8*a* shows the BTT of a sequence of 15-sec interval 800× macrophage images with one time slice image shown in Figure 8*b*. The BTT, derived from cell boundaries (in this case hand drawn), may be thought of as the two-dimensional probability distribution of the cell boundary. The quantitative measures of cell motion open the possibility of improved estimates of cytotoxicity, measures which are not dependent on cell death.

Despite the functional and morphologic changes, we have observed mitosis in asbestos-bearing cells so that the cytoskeletal assembly/disassembly mechanisms are at least partially intact.

Some of the changes in proteins found in the two-dimensional gels may be associated with mor-



a b

FIGURE 8. (*a*) Boundary trace transform (BTT) of 233 15-sec interval boundaries of a macrophage, (*b*) single instance of a macrophage.

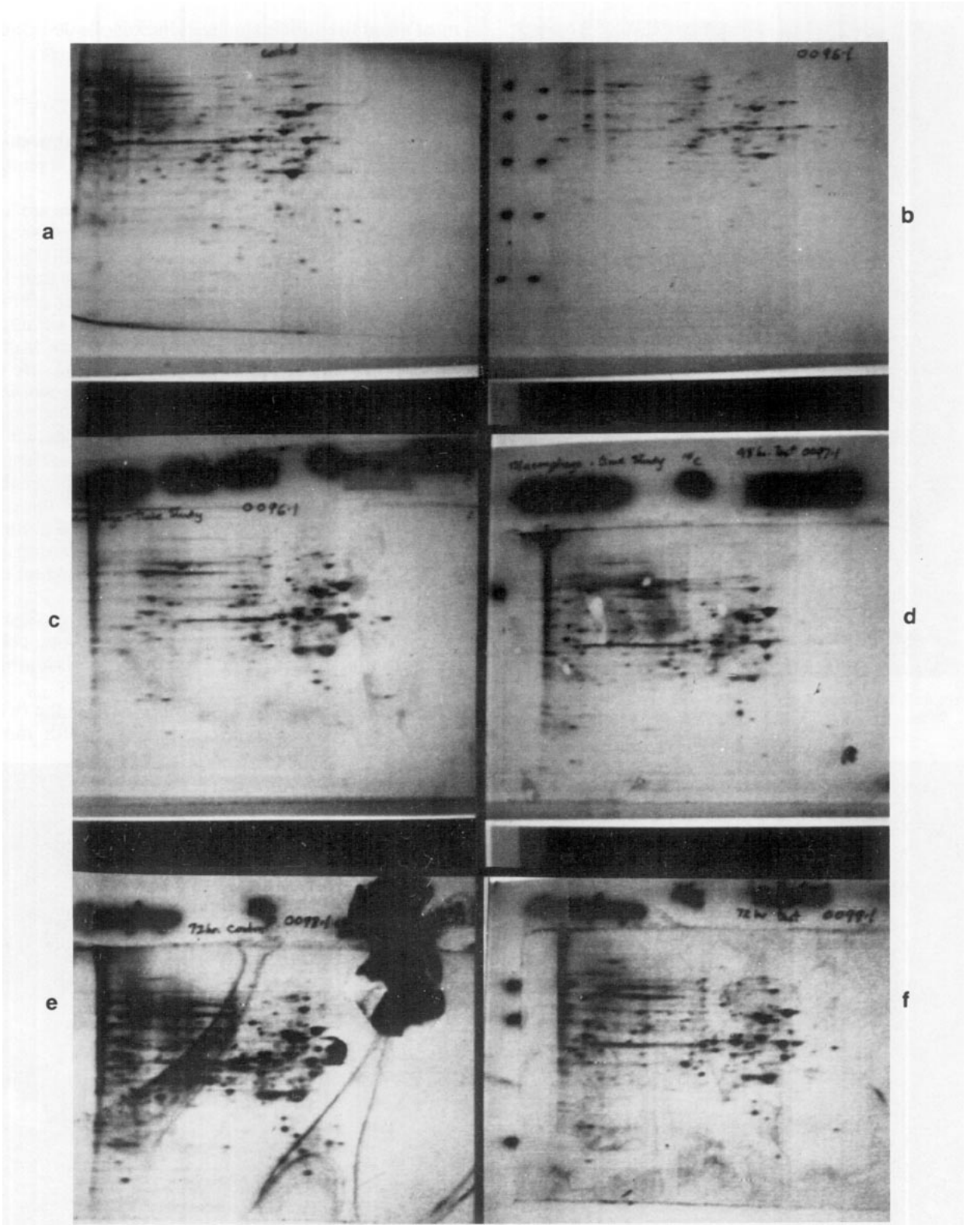


FIGURE 9. 1× of time series of macrophages challenged with amosite: (*a*) gel 94.1, 24 hr control; (*b*) gel 95.1, 24 hr amosite; (*c*) gel 96.1, 48 hr control; (*d*) gel 97.1, 48 hr amosite; (*e*) gel 98.1, 72 hr control; (*f*) gel 99.1, 72 hr amosite.

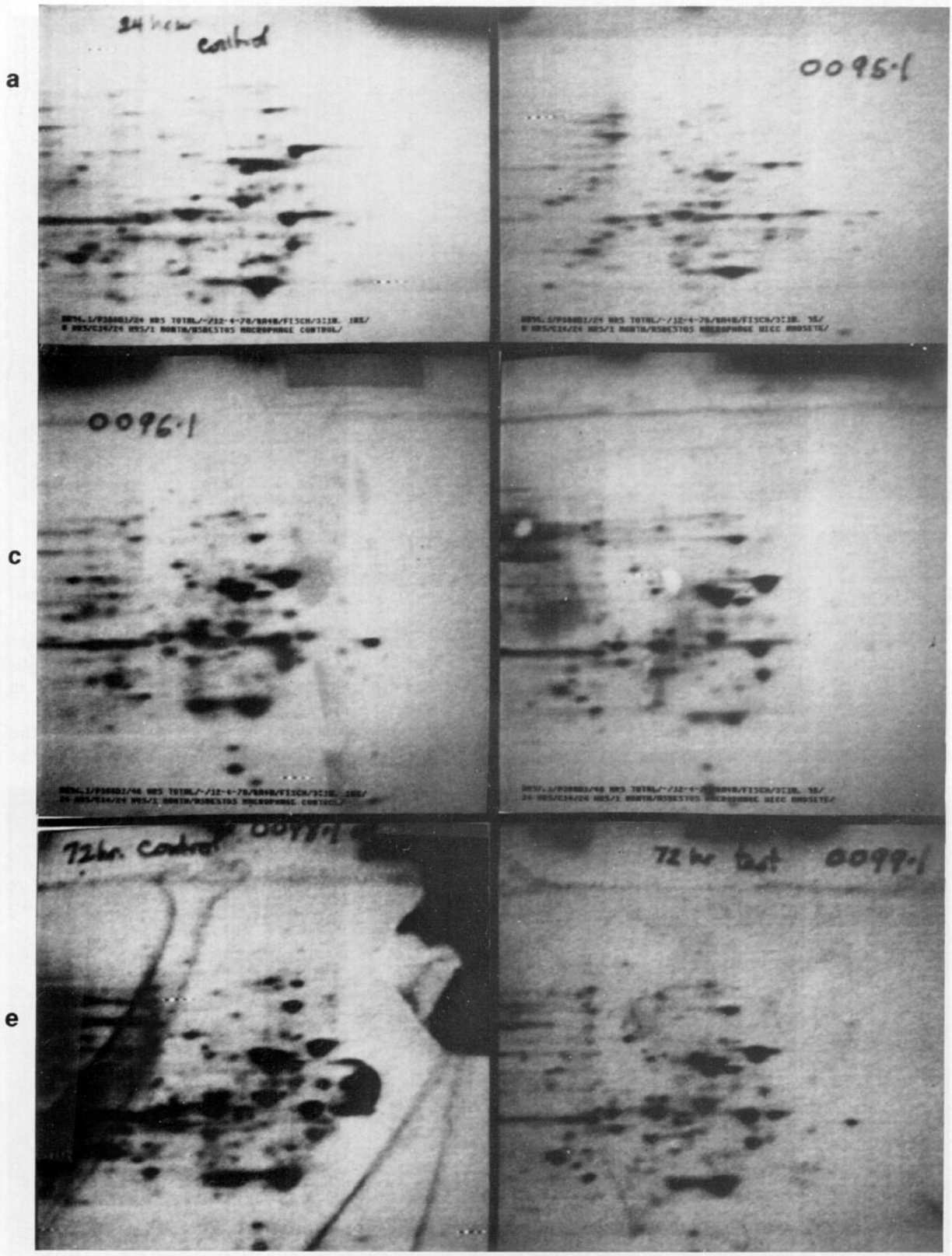


FIGURE 10. 2× of time series of macrophages challenged with amosite, major spot region: (*a*) gel 94.1, 24 hr control; (*b*) gel 95.1, 24-hr amosite; (*c*) gel 96.1, 48 hr control; (*d*) gel 97.1, 48 hr amosite; (*e*) gel 98.1, 72 hr control; (*b*) gel 99.1, 72 hr amosite.

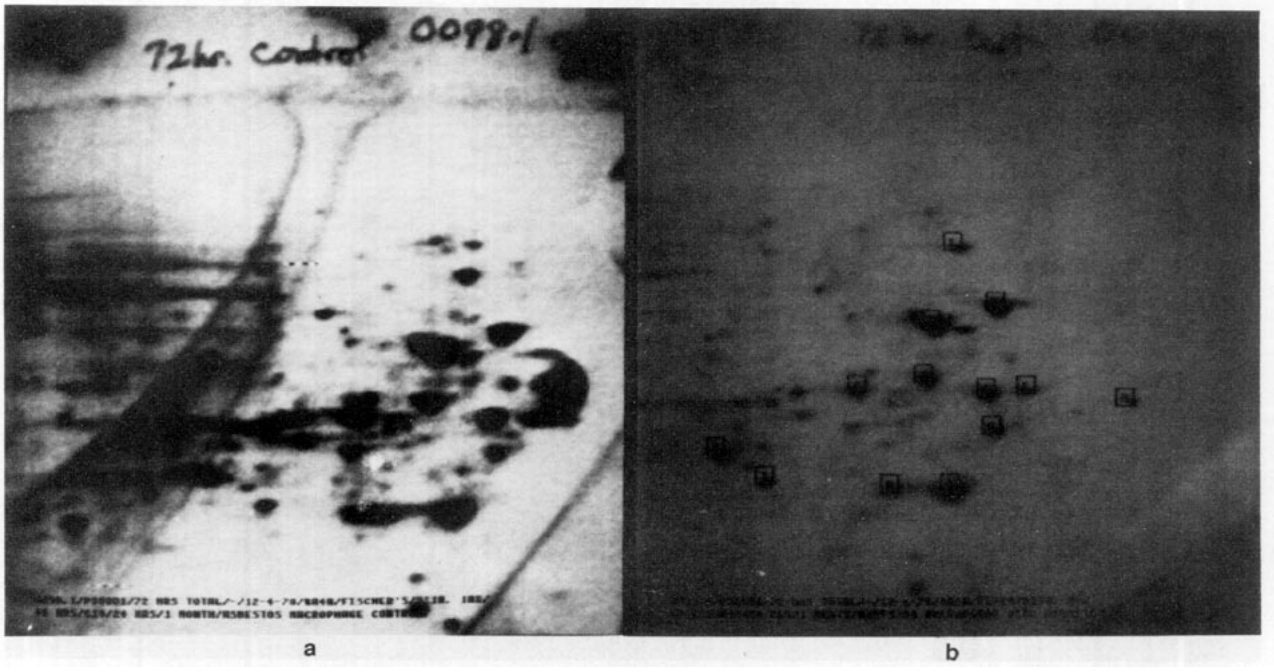


FIGURE 11. Pair of gels (72 hr) where the actin complex is most dissimilar: (*a*) control gel 98.1, (*b*) amosite gel 99.1, 2×.

phological changes in the macrophage. We have not yet identified these structural proteins in the gel.

Results

Several gels were run for both control and amosite asbestos with asbestos added 24, 48, and 72 hr before harvesting. These gels are illustrated in Figures 9 and 10, where gels 94.1, 95.1 are the 24 hr control and amosite runs, 96.1, 97.1 are the 48 hr runs, and 98.1, 99.1 are the 72 hr runs. The protein with a molecular weight of about 45,000 seems to consist of two major spots which sometimes merge together and may in fact be a single protein which differs by charge.

Figure 11 shows the control (Fig. 11*a*) and the amosite (Fig. 11*b*) gel after 72 hr. Notice that the

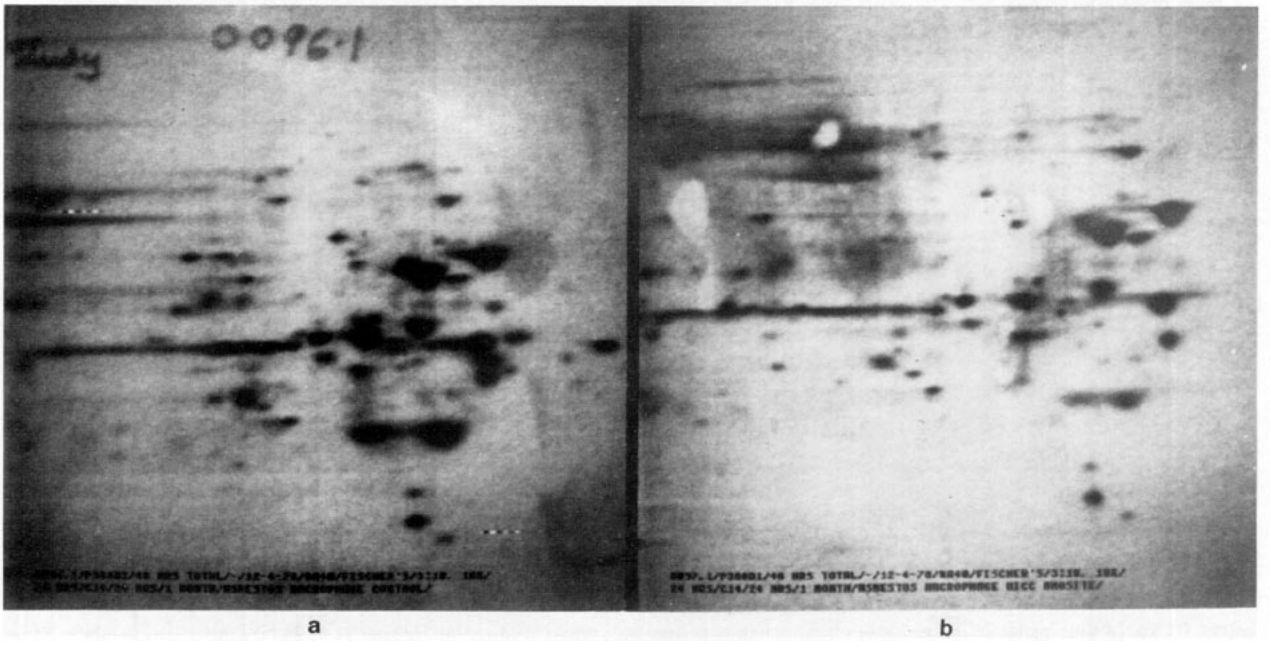


FIGURE 12. Major spot differences between control and amosite at 48 hr: (*a*) control gel 96.1; (*b*) amosite gel 97.1; 2×.

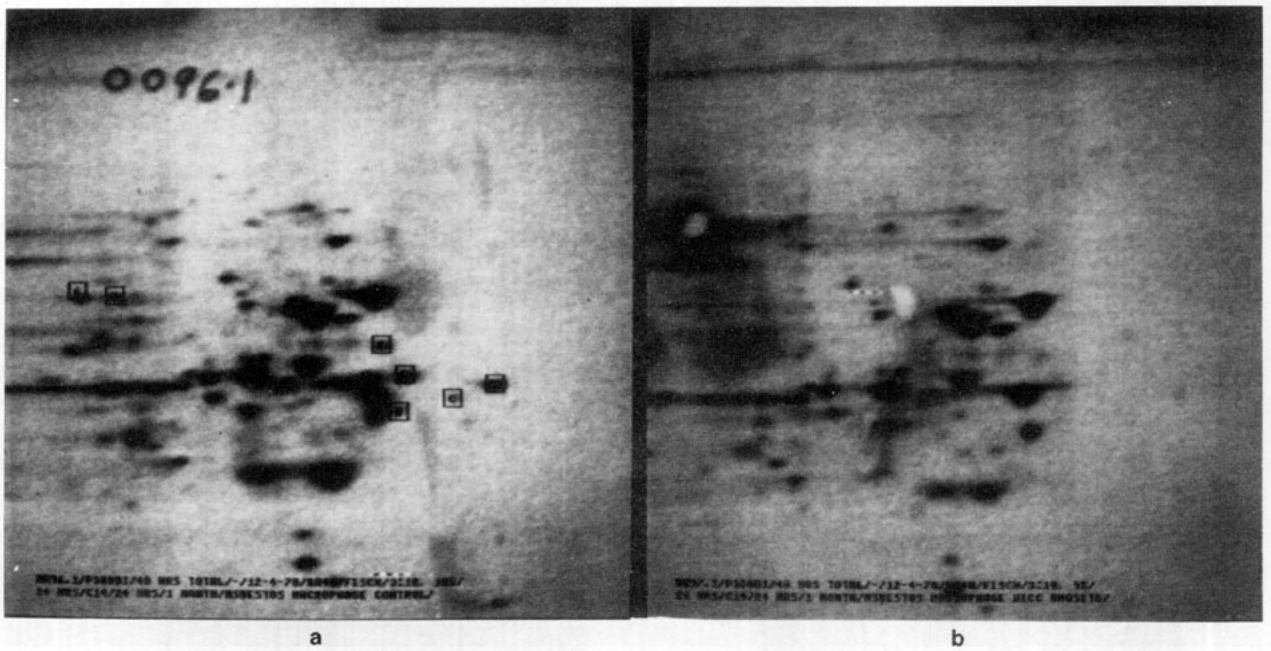


FIGURE 13. C-map overlay image of (*a*) control gel of 96.1, (*b*) corresponding region in amosite gel 97.1: 2×.

leftmost actin spot seems to be considerably lighter in the amosite gel. Preliminary surveys of electrophoretograms suggest obvious quantitive and qualitative differences between control and asbestos treated cells. However, comparisons of gels are hindered by a number of problems: the number of cells, and cellular protein obtained from each experiment is affected by the cytotoxic effect of asbestos, these variations make it difficult to use equal protein concentrations in each gel, it is also difficult to label the cellular proteins to the same specific activity with radioactive amino acids. These problems are compounded by variables in sample processing, electrophoresis, autoradiography and film development. We are in the process of repeating these experiments with more extensive controls as well as extending these experiments to effects of other durable fibers.

Major Spot Differences

Major spot differences were noted in the 48 hr control and asbestos gels, as is seen in Figure 12. The spots present in the control gel but missing in the asbestos gel (Fig. 13*b*) were marked as shownin Figure 12*a*. Figure 14 shows a canonical spot map (C-map) illustrating the pattern of these spots for the gels in the time series. Canonical spots (C-spots) A through E intially seemed indicative of a major difference depending on fiber phagocytosis. These effects were not observed in the 24-hr and 72-hr samples. The following notation is used in C-map tables: • = not tested; + = present; − = missing; ? = not sure whether + or −; P or (+?) = probably present; M or (−?) = probably missing; R = right shift; D = downward shift; U = upward shift; D = downward shift; L = left shift; B = blacker in density; W = whiter in density; T = two or more spots (i.e., multiple spots). A canonical spot, C-spot, is defined as a 4-tuple:

⟨2 letter spot code⟩, ⟨acc#⟩, ⟨search group⟩, ⟨x, y coordinates⟩

Protein Densitometry Analysis

Having noticed the density difference in the left spot of the 45,000 Dalton protein we decided to perform density measurements on this set of proteins and other major spots in the set of gels. In nonsaturating gels, the total density of an autoradiography spot has a fairly linearly correspondence to the amount of protein present. Several spots were selected in addition to the 45,000 Dalton protein which appeared present in all of the gels. Figure 15 shows the spots selected to be measured. The left most spot is labeled *A* and the right labeled *B*.

The analysis of the spot data is preliminary and requires additional experiments before it can be reliably interpreted. However, some preliminary results are presented. Figure 16 shows the time plot of the ratio of the left to the right spots in the actin complex:

C-SPOT		S_{35} 8 days C	S_{35} 8 days A			C_{14} 24 C	C_{14} 24 A	C_{14} 48 C	C_{14} 48 A	C_{14} 72 hrs C	C_{14} 72 hrs A
		0	0	0	0	0	0	0	0	0	0
		0	0	0	0	0	0	0	0	0	0
		7	7	9	9	9	9	9	9	9	9
		6	7	2	3	4	5	6	7	8	9
		.	.	.	.	.	.	.	.	.	.
		1	1	1	1	1	1	1	1	1	1
A-0096.1ASBCA360	237	P	-	.	.	-	-	+	-	M	P
B-0096.1ASBCA369	243	+	+	.	.	P	+	+	-	W	+
C-0096.1ASBCA390	237	+	+	.	.	+	P	+	-	?	+
D-0096.1ASBCA407	231	+	+	.	.	-	P	+	-	?	+
E-0096.1ASBCA371	228	P	P	.	.	+	+	+	-	?	+
F-0096.1ASBCA362	216	+	+	.	.	+	-	+	W	+	+
G-0096.1ASBCA238	193	+	+	.	.	+	+	+	M	+	+
H-0096.1ASBCA253	195	+	+	.	.	+	M	+	M	+	+
I-0096.1ASBCA343	231	+	P	.	.	-	-	+	M	-	-
J-0096.1ASBCA346	160	M	?	.	.	P	?	+	P	B	P

C-SPOT		S_{35} 8 days C	S_{35} 8 days A			C_{14} 24 C	C_{14} 24 A	C_{14} 48 C	C_{14} 48 A	C_{14} 72 hrs C	C_{14} 72 hrs A
		0	0	0	0	0	0	0	0	0	0
		7	7	9	9	9	9	9	9	9	9
		6	7	2	3	4	5	6	7	8	9
		.	.	.	.	.	.	.	.	.	.
		1	1	1	1	1	1	1	1	1	1
A-0099.1ASBDN306	250	+	+	.	.	+	+	+	+	+	+
B-0099.1ASBDN330	250	+	+	.	.	+	+	+	+	+	+
C-0099.1ASBDN293	210	+	+	.	.	+	+	+	+	+	+
D-0099.1ASBDN320	206	+	+	.	.	+	+	+	+	+	+
E-0099.1ASBDN345	212	+	+	.	.	+	+	+	+	+	+
F-0099.1ASBDN361	211	+	+	.	.	M	W	+	?	M	+
G-0099.1ASBDN347	227	+	+	.	.	+	+	+	+	+	+
H-0099.1ASBDN400	216	+	+	.	.	+	+	+	+	+	+
I-0099.1ASBDN332	153	+	+	.	.	+	+	+	+	+	+
J-0099.1ASBDN255	246	?	W	.	.	+	+	+	+	+	+
K-0099.1ASBDN323	184	+	+	.	.	+	+	+	+	+	+
L-0099.1ASBDN349	177	+	+	.	.	+	+	+	+	+	+
M-0099.1ASBDN235	234	?	W	.	.	+	+	+	+	+	+

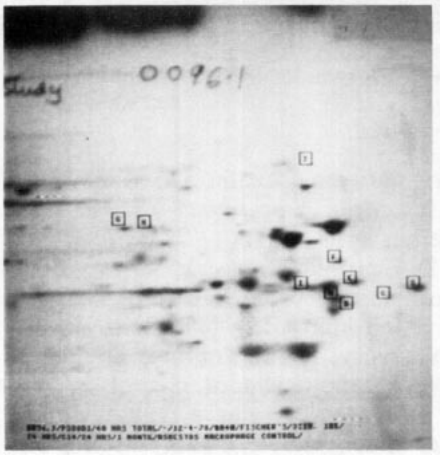


FIGURE 14. Printout of C-map for canonical spots in control gel 96.1.

FIGURE 15. C-map of amosite gel 99.1 spots selected for making density measurements at 2×.

A/B. Notice the spread in the 72-hr sample. Figure 17 shows the relative stability of spots *E* and *G* relative to *D* over time and between control and asbestos. Figure 18 shows the variability of ratios *A/D* and *B/D*. This might imply that the turn-over rates for spots *A* and *B* do not seem to be changing the same way as for spots *D*, *E*, and *G*.

Rank Ordering of Spots

The rank ordering of unnormalized spot densities (suggested by E. Lester) within a gel is interesting, in that it shows qualitative shifts in the relative concentrations of polypeptides over time. Figure 19 shows the rank order of the 13 spots in the control gels, and Figure 20 the rank ordering in the amosite gels. Spots *F* and *H* were missing data in some of the gels and should be discounted for now. In both control and amosite, the *B* spot is greater spot *K* at 24 hr, but *K* at 24 hr, but *K* > *B* at 72 hr. In both control and amosite gels at 48 hr, there is a major drop in spot *B* relative to spots *K* and *L*. Spot *A* is less than spots *D*, *C*, *L*, *E*, *K*, *B* in the 24 hr control but only less than *B*, *K* in the 72-hr control. In the amosite 24-hr sample, spot *A* is less than *D*, *L*, *E*, *F*, *C*, *K*, or *B*, while at 72 hr it is less than *C*, *D*, *E*, *L*, *B*, or *K*.

The difficulties in interpreting these data can be appreciated in the next graph. A spot may be normalized by the sum of the spots present in the gel where it is measured. For a large number of spots this sum would approximate the total amount of material. This normalization is less valid for a small number of spots. Figure 21 shows the plot over time of the ratios of the asbestos to control normalized densities for all 13 spots *A* through *M*. Although evidence seems to

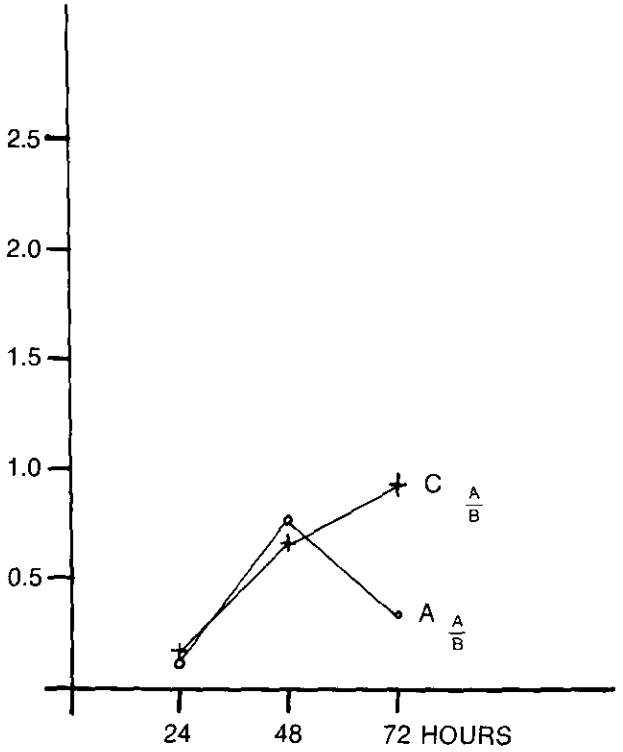


FIGURE 16. Ratio of spot densities *A/B* within each gel over time.

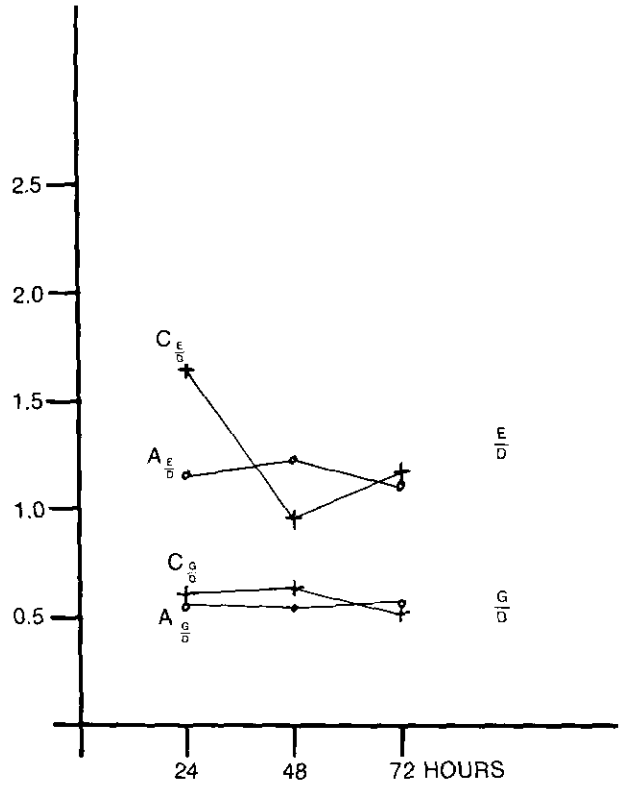


FIGURE 17. Ratio of spot densities *E/D* and *G/D* within each gel over time.

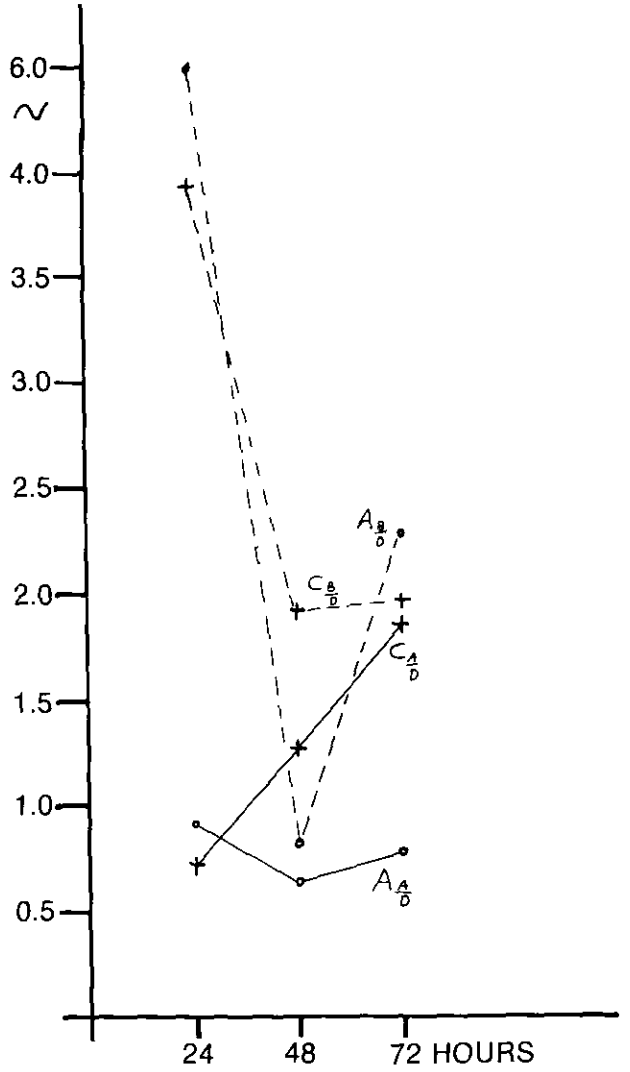


FIGURE 18. Ratio of spot densities *A/D* and *B/D* within each gel over time.

show changes in actin, these are not yet completely clear. This is illustrated by the many polypeptide concentration changes in both directions (increasing and decreasing) seen in Figure 21.

Comparing Spots Between Gels Automatically

The automatic segmentation and comparison of most of the hundreds of spots in a set of gels would seem to be a necessary condition in order to make sure that subtle changes in a cell system are not being missed. We are currently implementing programs toward this goal.

Figure 22 shows the results of comparing the spot lists from two previously computer-segmented

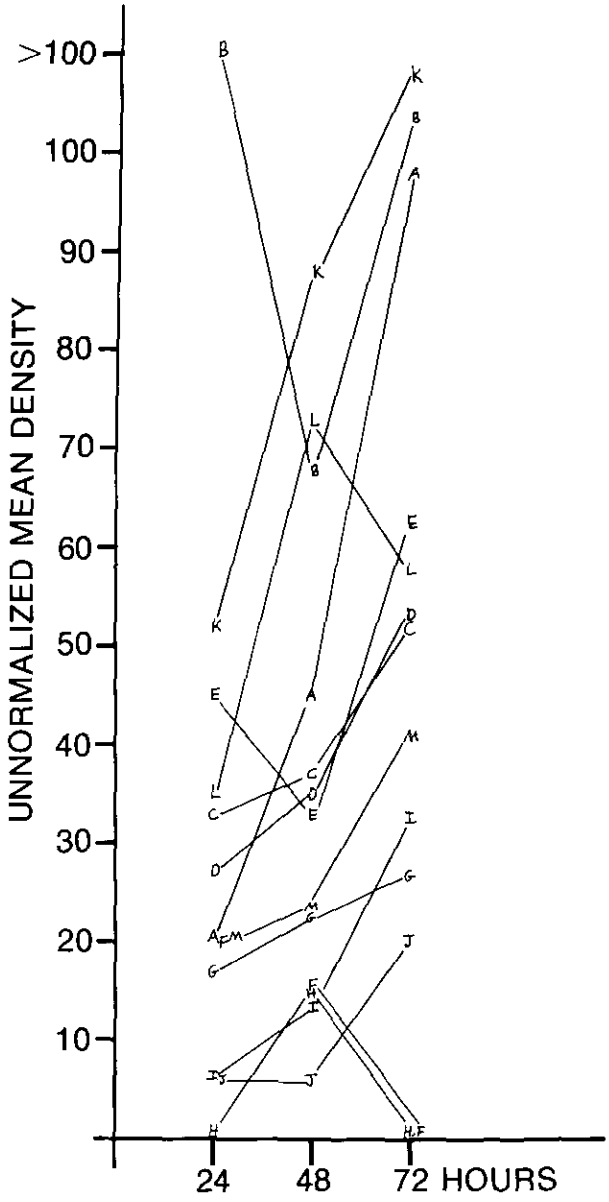


FIGURE 19. Rank ordering of spots *A* through *M* in the three control gels.

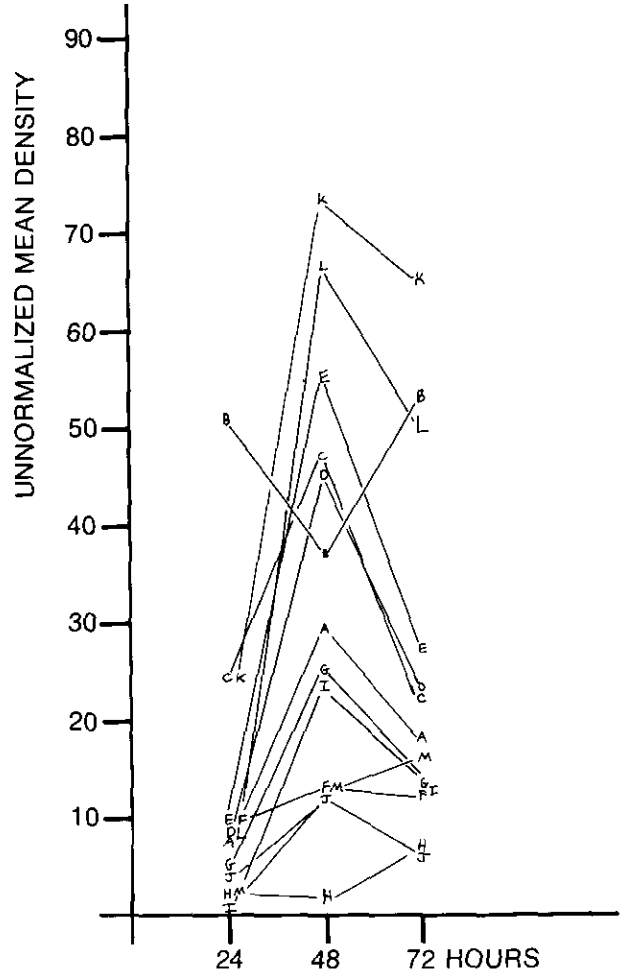


FIGURE 20. Rank ordering of spots *A* through *M* in the three amosite gels.

P388D₁ gels. The like cells were grown for 8 days and labeled with S35 rather than C14.

Each gel image was segmented by using multiple segmentations at increasing denser spot thresholds. Spots found during a pass which meet a spot morphologic criterion are accepted and removed from the image. The resultant list of spot positions and features constitutes the spot list. Two gels which are to be compared are then flickered and a set of from 10 to 25 easily located "landmark" spots are manually marked (denoted by squares on in Fig. 22). Each gel spot list is then partitioned into these landmark sets. Spots within each corresponding landmark set are then more easily and reliably compared. The heuristic assigns "sure pair" to well matched spots meeting a minimum-distance criterion, "possible pair" to those not as well matched or greater than a certain distance from the landmark set. "Ambiguous spots" are defined as those for which an adjacent spot makes a better match. Unresolved spots are those not meeting the minimum pairing criterion. The spots are labeled U (unresolved spot), P (possible pair), A (ambiguous pair), and S (sure pair).

The large number of unresolved spots (U labels) in the control sample is partly due to increased material in the control (relative to the asbestos sample due to cytotoxicity discussed before) as well as inadequacies of the current segmentation program to pick up very light spots in the gels. Further work is in progress to improve the segmentation as well as the spot pairing methodology which will make such comparisons more useful.

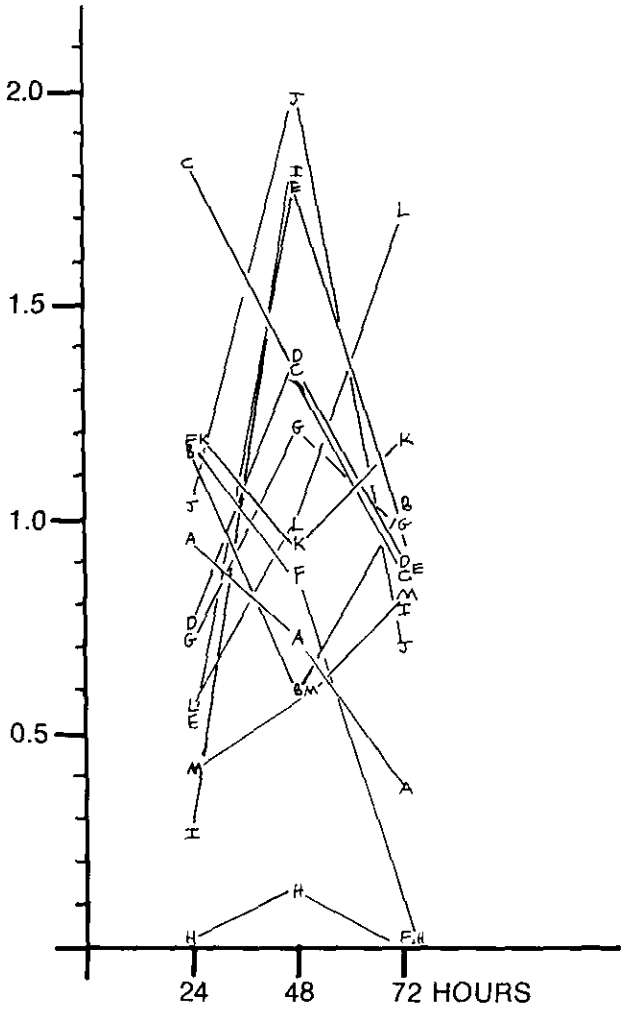


FIGURE 21. Ratios of normalized spot densities for amosite/control spots *A:M*.

Discussion

Although we are able to find spot differences which appear to be indicative of asbestos phagocytosis, we are dependent upon biochemical and/or immunologic help to actually identify the proteins corresponding to these spots. This will be necessary to probe further the nature of the asbestos cellular lesion.

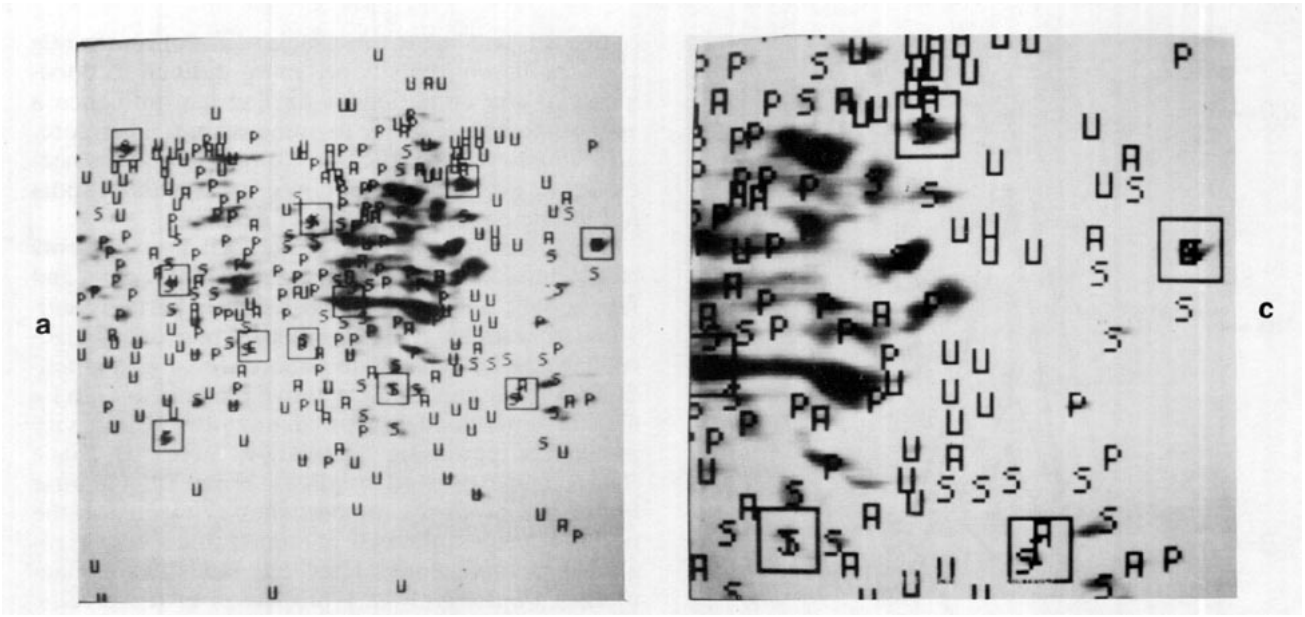
The problem of quantitating spot differences has arisen repeatedly in our collaborative study of lymphocyte proteins from Alzheimers disease patients and their family members (Merril, Shifrin, Ebert, Polinsky, Lemkin, and Lipkin, unpublished observations). Problems occur as to the identity of spots, and variations due to sample and gel preparation tend to obscure spot differences. For those changes which are obvious, simply using a flicker comparison of two gels will locate these major differences. Subtle changes in spot density are more difficult to document, as the condition of the gel can influence a spot's apparent density to some extent. Therefore, an understanding of the range of gel samples is necessary to extract maximum information about spots in a particular gel or subset of gels.

The P388D$_1$ macrophagelike cell line presents many characteristics which are morphologically and functionally analogous to those seen in partially activated macrophages (e.g., mouse peritoneal macrophages). These include adherence to glass, dendritic cellular appearance, active phagocytosis, a Fc marker, increasing lysomal enzymelike activity paralleling increasing "activation," etc. (*5*). Since this cell line derived originally from DBA mouse tumor still produces tumors when reinjected in the original species, the cell line must of course be regarded as malignant. They are not "ture" macrophages but have many properties of these cells. They are widely used by immunologists and other investigators for these properties.

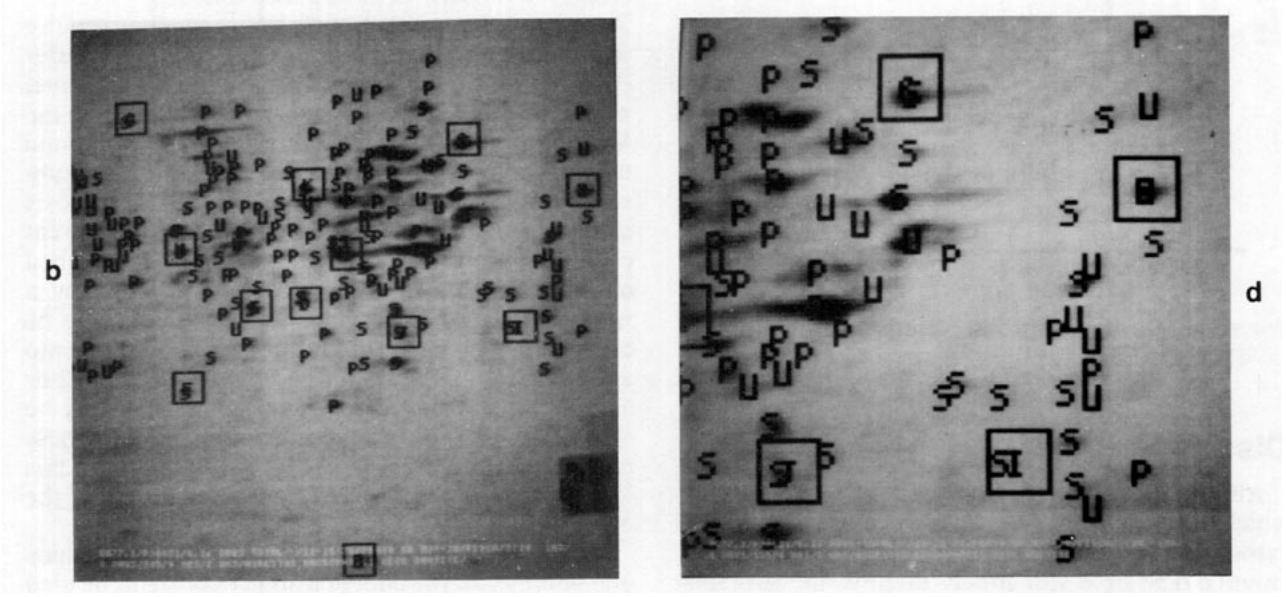
The advantages of P388D$_1$ cells for asbestos research have been reviewed elsewhere (*3*). The exigencies of the present bioassay procedure require that there be change in medium at about 48 hr after the initiation of the procedure. This, as noted above, will result in some cell loss, especially of cells in the M phase of the cell cycle, since it is these that round up and temporally lose their adherance to the substrate. We have shown elsewhere that cells in mitosis in fact bear fibers so that the loss of nonadherent cells entails a concommittant fiber loss. Unpublished observations on P388D$_1$ cell cycle times allow a rough estimate of the upper limit of this loss to be computed at about 10%. This loss must be taken into account when quantitating protein changes at 72 hr after fiber exposure. The loss though small may be significant for some proteins and may also make less obvious some increases observed. In any case this loss of cells and fibers cannot account for all of the observed changes.

Fiber concentrations of 100 $\mu g/cm^3$ do not produce enough cells for the concentration of proteins needed to run the two-dimensional gels. The solution is either (a) to grow more cells, or (b) to use a more sensitive protein indicator, such as a silver stain for these gels (*13-14*); the latter is now being attempted on the macrophage cultures with encouraging preliminary results. Additional experiments include attempting to pulse label 8 hours before labeling.

The combining of such powerful techniques as automatic segmentation and comparison which an overall gel data management system will facilitate the analysis and compilation of spot and spot cluster statistics for large numbers of gels. We are currently



0076.1/P388D1/8.16 DAYS TOTAL/-/11-15-78/?#A39 OR A37-9/FISCH/3:10, 10%/
8 DAYS/S35/4 HRS/2 WKS/ASBESTOS MACROPHAGES CONTROL/



0077.1/P388D1/8.16 DAYS TOTAL/-/11-15-78/?#A39 OR A37-10/FISCH/3:10, 10%/
8 DAYS/S35/4 HRS/2 WKS/ASBESTOS MACROPHAGES UICC AMOSITE/

FIGURE 22. Labeled output from the gel comparison program CMPGEL: (*a*, *b*) control gel 76.1/amosite gel 77.1 at 2×; (*c*, *d*) control gel 76.1/ amosite gel 77.1 at 4×.

reaching the upper limits of the capacity of the CPU and disk storage of the RTPP (a PDP8e). With the expected addition of new CPU and disk storage capacity of a medium size computer, to be connected to the RTPP, the more complete analysis of a larger number of gels seems feasible.

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