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A Two-Dimensional Electrophoretic Analysis of the Heat-Shock-Induced Proteins of Human Cells

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Using two-dimensional electrophoresis, we have investigated the responses of human cells in culture to heat shock and to various chemical agents producing a similar effect. These treatments result in the induction of increased synthesis of several specific proteins. One (HShock:1, SDS-molecular mass about 65 000) is increased by about 350-fold over the amount in untreated cells. Computer analysis of time-course studies indicates, however, that rates of synthesis of various proteins other than the classical "heat shock proteins" are affected, some of these alterations following time courses quite different from the main (HShock) inductions. The heat shock effect is thus much more complicated than previously realized. We purified the HShock:1 protein from heat-shocked human lymphoblastoid cells, and prepared a rabbit antiserum specific for HShock:1 on nitrocellulose two-dimensional gel transfers of total lymphoblastoid cell protein.

A survey of mouse tissues shows high concentrations of an HShock:1-like protein in the testis, and human testes also appears to contain substantial (though lower) concentrations. These results are consistent with the hypothesis (derived from the tissue-culture studies) that the heat shock effect is a general response to the need for increased protein catabolism within the cell. Increased concentrations of HShock:1 are also observed in preparations of blood leukocytes collected from patients after surgery, indicating that some types of physiological trauma may induce the heat shock proteins in man. Using the antiHShock:1 antibody in an immunoassay, it will be possible to systematically examine HShock:1 concentrations in plasma and leukocytes, thereby opening up the possibility of a clinical test based for the first time upon an inducible aspect of cellular gene expression.

Additional Keyphrases: *effects and evaluation of stress · intensified catabolism of protein · TYCHO system · response to cell damage vs cell-death effects · leukocytes · lymphoblastoid cells · fibroblasts · testicular proteins · mouse spleen-cell proteins · ISO-DALT system · inter-species comparison · genetics of protein production · temporal aspects of response*

The response of cells to heat shock constitutes one of the most basic regulatory mechanisms to be found in living organisms. While the earliest observations of specific heat-shock effects concerned chromosome puffs in *Drosophila* salivary glands (*1*, *2*), it has become evident in the last five years that analogous sets of proteins can be induced in a wide variety of animal cells [human (*3–5*), chicken (*6*, *7*), salmon (*8*), and *Drosophila* (*9*)] by heat shock and by various chemical agents. Most frequently the induced proteins are quoted as having sodium dodecyl sulfate (SDS) molecular masses in the ranges (*a*) 83 100–100 000; (*b*) 68 000–76 000, and (*c*) 25 000–35 000 daltons. Induction of a specific set of such proteins has been observed in cultured chick-embryo fibroblasts after heat shock (*6*), or treatment with sulfhydryl reagents (*3*), transition metals (*3*), antabuse (*5*), sodium arsenite (*7*), puromycin (*4*), or amino acid analogs (*4*, *6*). Under conditions of extreme but survivable treatment, the synthesis of heat-shock proteins can increase quickly from very low proportions to a point at which they are temporarily the major protein products of the cell. Recent studies in the rat have demonstrated that such a temporary increase can occur in vivo (*10*) as well as in cultured cells, and hence that the relationship of the induced proteins to fever and to the effects of certain toxic chemicals may be important in man.

Our first experience with "heat-shock" proteins involved their appearance in two-dimensional gel electrophoresis patterns of human lymphocytes treated with a sample of a commercial prostaglandin E standard. It later became apparent that the large effects produced by this treatment were not due to prostaglandins, but rather to a contaminant, and that iodoacetamide (a sulfhydryl poison), heat shock, or cadmium treatment could produce similar increases in the synthesis of a few specific proteins. We also noted that these proteins were occasionally increased in lymphocytes obtained directly from human blood, and hence that an understanding of the regulation system responsible would be important in the analysis of lymphocyte proteins in both research and clinical settings. Because heat shock is the best studied of the various treatments inducing the effect, we have designated the proteins "HShock:1–n," in accordance with the protein-set nomenclature convention we have devised (*11*, *12*).

In this paper, we have carefully attempted to delineate the proteins involved in the heat-shock response in human lymphoid cells through the use of high-resolution two-dimensional electrophoretic protein mapping. Quantitative analysis of the two-dimensional patterns with the TYCHO computer system (*12*, *13*) has allowed us to investigate the time course of the effect, and to show that synthesis of a variety of proteins not generally considered heat-shock proteins is affected to some extent by the heat shock. Using the gel-based Protein Index approach (*12*, *14*, *15*), a variety of other experimental methods has also been brought to bear on the problems of characterizing the heat-shock proteins and investigating their possible functions in the cell.

As a potential clinical indicator of certain types of cellular damage, the heat-shock proteins are unique: appropriate damage causes a dramatic increase in the synthesis of these proteins, rather than merely causing their release from the tissues into the blood. Thus, there is an opportunity to measure a responsive aspect of gene expression itself, instead of the degree of leakiness of affected cells.

Materials and Methods

Isolation, Growth, and Treatment of Cells

Fresh human mononuclear leukocytes were prepared by the Ficoll–Paque procedure (Pharmacia Fine Chemicals, Piscataway, NJ 08854) as previously described (*12*). Human GM607 lymphoblastoid cells (from a normal individual; obtained by us from the Human Genetic Mutant Cell Repository, Camden,

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NJ 08103) were grown in RPMI 1640 medium as previously described (*11*). The normal human fibroblast cell line designated 1494 (obtained with the help of Dr. William Blattner from Meloy Labs, Inc., Springfield, VA 22151; NCI contract no. NO1-CP91000) was maintained in Dulbecco's Modified Eagle's Medium. Fibroblastic or epithelial cells lines from a variety of species were obtained from the American Type Culture Collection, Rockville, MD 20852, and their respective HShock:1 proteins were identified after induction with iodoacetamide (1 μg added to the growth medium per milliliter during a 24-h labeling period). All cells were labeled in RPMI 1640 medium lacking methionine under conditions previously described (*11*, *12*).

GM607 cells were heat shocked by gentle shaking in a small volume of medium in a 75-cm^2 tissue-culture flask placed on the platform of a shaking waterbath at 45 °C for intervals ranging from 2 to 30 min. The flask was slightly immersed in the water, to facilitate heat transfer. After heat shock, the cells were transferred to multiwell plates and maintained in an incubator [CO_2/air (5/95 by vol), 37 °C, 95% relative humidity] until and during labeling with [^{35}S]methionine.

Sample Preparation and Two-Dimensional Electrophoresis

Cells were solubilized directly in pH 9.5 urea sample buffer containing, per liter, 9 mol of urea, 20 mL of NP-40 detergent, 10 mL of mercaptoethanol, and 20 mL of pH 9–11 ampholytes (LKB Instruments, Rockville, MD 20852). Testicular samples were solubilized by the methods developed by Giometti et al. (*16*) for frozen sections of muscle biopsies.

Two-dimensional electrophoresis was performed with the ISO-DALT system (18 × 18 cm format) as previously described (*17*, *18*). All samples were co-electrophoresed with creatine kinase (EC 2.7.3.2) charge standards (*19*). Gels were either fluorographed (*20*) or dried directly under reduced pressure, and autoradiographed or fluorographed on Kodak XAR-2 X-ray film. Nitrocellulose transfers of two-dimensional patterns were prepared and stained with antibody according to procedures described (*21*, *22*).

Computer Analysis of Two-Dimensional Gel Data

A series of gels from time-course studies of the heat-shock effect were analyzed by using the TYCHO I system previously described (*12*). Values obtained with this system are generally found to agree within about 10%. The data from different gels were scaled together by taking as reference the integrated densities of spots known to represent mitochondrial proteins Mitcon:1 and Mitcon:3 (*11*), the synthesis rates of which were judged to be constant throughout the experiment.

The nomenclature used here for the major heat-shock proteins (the six-letter mnemonic "HShock," followed by a serial number for each member of the set) follows our current practice in naming specific protein sets (*11*, *12*). Although other treatments besides heat shock induce these molecules, the name HShock is preserved for historical reasons.

Isolation of Human HShock:1, Preparation of Specific Antibody

HShock:1 was isolated from batches of GM607 human lymphoblastoid cells (each ~5 × 10^8 cells) one day after 15-min heat shock at 45 °C. The cells were washed three times in phosphate-buffered isotonic saline and mechanically homogenized in 2 mL of pH 6.2 column-equilibration buffer (containing, per liter, 25 mmol of citrate, 1 mmol of EDTA, and 0.2 g of NaN_3) with 1 mg of mercaptoethanol per milliliter. This extract was centrifuged at 140 000 × *g* for 30 min, and the pellet was discarded. The extract was then applied to a 1.5 × 35 cm column of DEAE-Sepharose CL-6B (Pharmacia), which had been equilibrated with and was eluted with the equilibration buffer described above. Fractions of the eluate were dialyzed against water and lyophilized, and part of the dry residue was resuspended in urea sample buffer and analyzed by two-dimensional electrophoresis. A volume corresponding to a single peak in the column eluate proved to contain most of the HShock:1 protein, in fairly pure form (>95%). Dutch Belted rabbits were given intradermal injections of this material in Freund's complete adjuvant at several sites, and then were given booster injections with similar material in Freund's incomplete adjuvant two and four weeks after the initial immunization. Specific antibody (as judged by specific staining of a nitrocellulose transfer of a two-dimensional gel of whole GM607 protein) was obtained at six weeks.

Results

Identification of Major Proteins Induced by Heat Shock

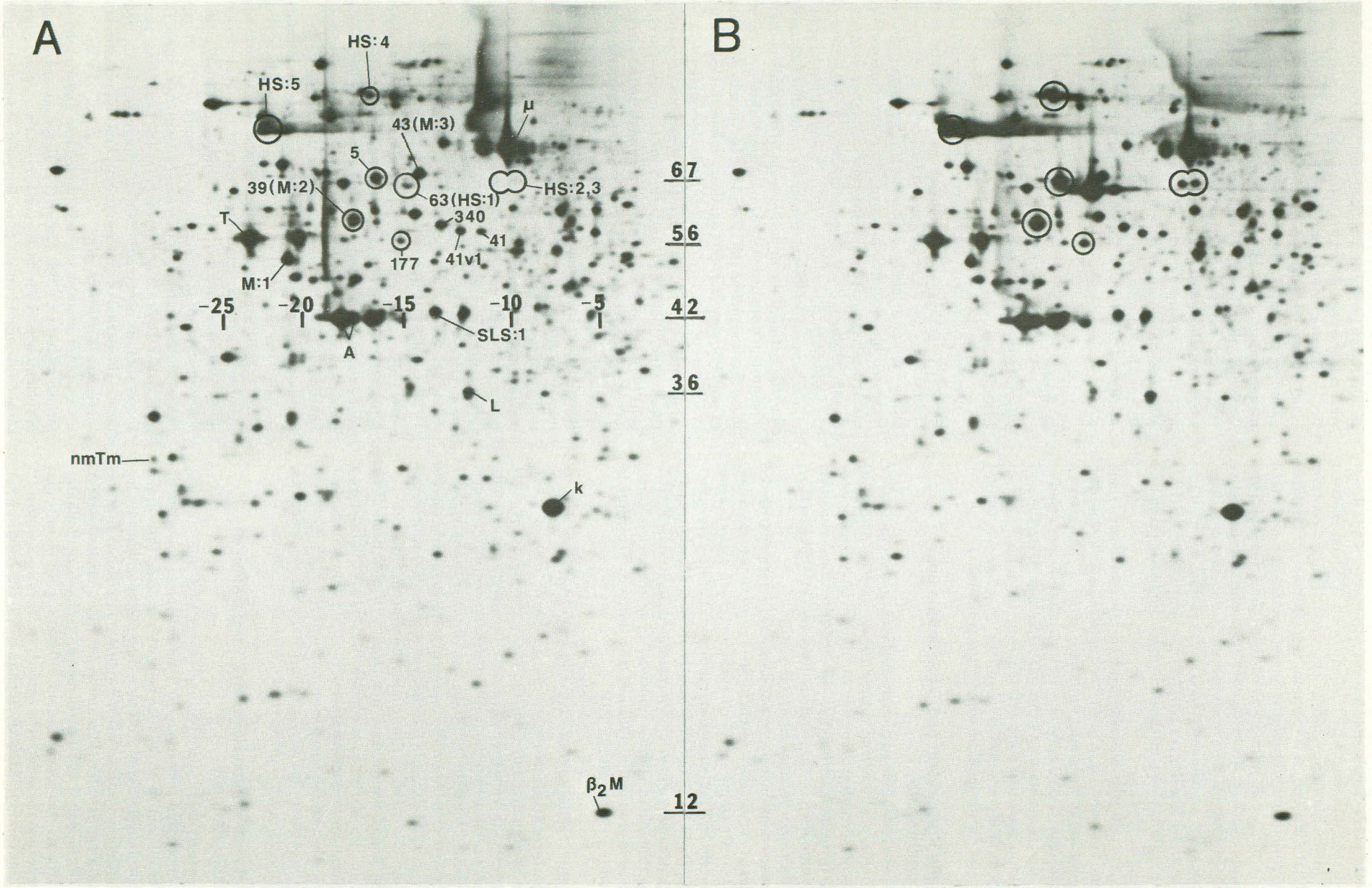
A set of five major proteins is induced in human lymphoblastoid cells within 2 h after a heat shock of 10 min at 45 °C (Figure 1). This set is designated HShock:1–5 (abbreviated HS:1–5 in figure labels). HShock:1 (the synthesis of which is increased by about 350-fold over the control amount) is the major heat-shock protein observed in higher animals. Although HShock:1 varies considerably in isoelectric point throughout the mammals (Figure 2), indicating a rapid rate of biochemical evolution, its relative molecular mass and the characteristics of its induction seem to be highly conserved. The protein designed MSN:5, in contrast, shows no variation in gel position among the species tested, thereby affording an example of very slow biochemical evolution. HShock:2 and :3 appear not to have easily detectable analogs in other mammalian species, although their positions are similar to those of the "thermin B" heat-shock spots observed in chicken cells (*23*). HShock:5 probably corresponds to the larger (M_r 80 000–100 000) heat-shock protein found in most mammalian cells, and HShock:4 may be another protein more easily detected in man than other species. So far, we have observed no human counterpart to the heat-shock protein of lower relative molecular mass (25 000–35 000) observed in other species.

Close examination of the differences between control and heat-shocked cells indicates that the synthesis of several other cellular proteins is significantly affected by heat shock, though not to the degree of the major HShock proteins.

Time Course of the Heat Shock Effect

By pulse-labeling cells at various times after heat shock, it is possible to observe how the heat-shock-induced changes in gene expression develop over time. Figure 3 shows a series of time points during the first 25 h of the response of human lymphoblastoid cells to a 30-min heat shock at 45 °C. The synthesis of HShock:1 rapidly increases to a maximum and then declines to a very low (possibly subcontrol) level (Figure 3*H–N*). Synthesis of other heat-shock proteins follows an apparently similar time course, except for a few transient molecules seen only in the first sample collected after the shock (downward pointing arrows in Figure 3*I*). Examination of the total amount of protein (stained with Coomassie Blue) rather than the rate of synthesis (at the same time points) shows that, as expected, HShock:1 increases in abundance during the period of maximum synthesis and remains stable thereafter (Figure 3*A–G*).

Computer analysis of the time-course experiments performed with the TYCHO system (*12*) allows quantitative study of such induction effects. Figure 4 shows the rates of synthesis of several individual proteins followed through time after


A
HS:4
HS:5
μ
43(M:3)
5
67
39(M:2)
63(HS:1)
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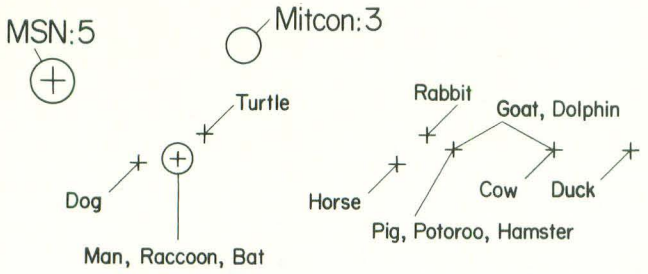

Fig. 2. Schematic diagram showing relative positions of the HShock:1 spot in cultured fibroblasts or epidermal cells from a variety of animal species

Circles show the positions of HShock:1 and (for reference) of Mitcon:3 (a mitochondrial protein), and the major protein with Master Spot Number 5 (MSN:5) in human cells. The position of MSN:5 from all the animals shown is identical to the position in man, while the position of HShock:1 (induced in these cells with iodoacetamide) varies considerably. Some species (goat, dolphin) appear to have two forms of HShock:1, perhaps because of heterozygosity in the individuals from which the cultured cell lines were derived

three different heat-shock treatments: 2, 10, or 30 min at 45 °C. For the major heat-shock proteins [exemplified here by HShock:1 (MSN:63)], it is apparent that both the time course and the maximum rate of synthesis depend on the severity of the initial heat shock. The results also demonstrate very clearly that proteins other than the classical heat-shock proteins follow the heat-shock induction profile, though with smaller total changes in rates of synthesis. Proteins designated by Master Spot Numbers (MSNs) 5, 39, and 177 show such clear, low-amplitude induction effects. The response of MSN:39 is particularly interesting, because it is a mitochondrial protein (Mitcon:2, ref. 11), none of which has previously been implicated in the heat-shock response. Other major mitochondrial proteins, exemplified by MSN:43 (Mitcon:3), are not similarly affected. Quantitative analysis also reveals several classes of proteins for which the rate of synthesis varies after heat shock, but not according to the usual induction profile; MSN:41 and 41vl exemplify a class whose synthesis decreases slightly late in the response, while SLS:1 shows an increase in synthesis late in the response.

Persistence of Heat-Shock Protein

If human lymphoblastoid cells are heat shocked and then maintained in culture for an extended period, HShock:1 (the only heat-shock protein clearly visible as a stained spot) declines gradually to control amounts by the fifth day (Figure 5*A–E*). This decline can be accounted for most simply by the dilution of the HShock:1 during a number of cell divisions, rather than by specific breakdown of the protein. In any case, the protein is stable within the cell for some days.

When cells are heat shocked repeatedly, once per day, the amount of HShock:1 can be maintained apparently indefi-

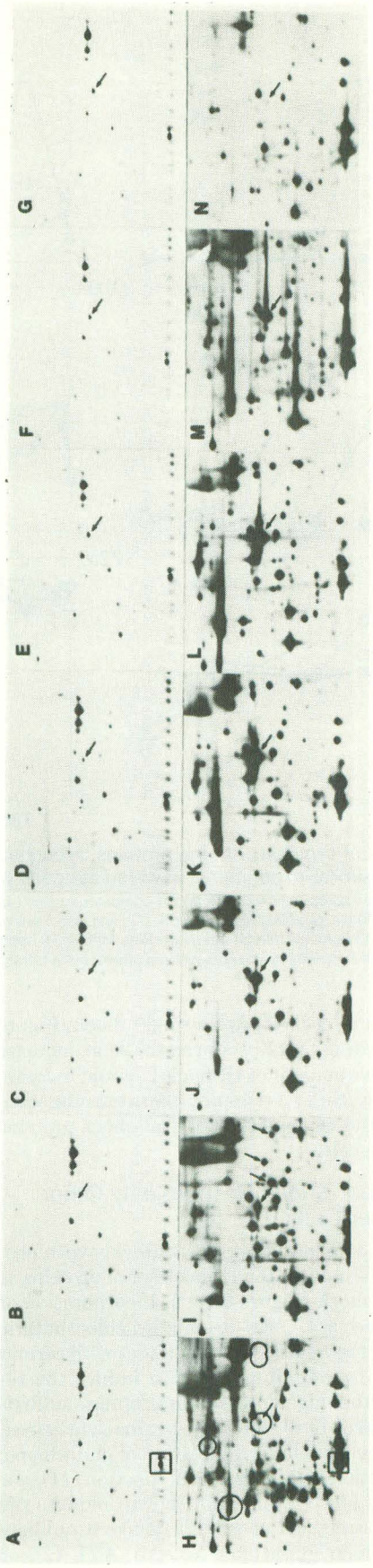

Fig. 3. Portions of two-dimensional gels, showing the time course of events in human lymphoblastoid cells (GM607) after a 30-min heat shock at 45 °C

Panels *A–G* are photographs of stained spots in patterns from cells solubilized at (*A*) 0, (*B*) 0.25, (*C*) 1.75, (*D*) 3, (*E*) 5, (*F*) 9, and (*G*) 25 h after the conclusion of the heat shock. They illustrate the total relative amounts of various proteins present at the time of solubilization. Panels *H–N* are fluorographs of the same gels, showing the amount of radioactive amino acid label incorporated into various proteins during a 30-min pulse label immediately before solubilization; thus these illustrate the rate of synthesis of the proteins. The horizontal rows of dots along the base of panels *A–G* are internal protein standards (*19*). *Squares* in panels *A* and *H* surround the spots of β and γ actin; *circles* in panel *H* indicate the positions of the major heat-shock proteins (as in Figure 1). *Upward pointing arrows* indicate HShock:1. *Downward pointing arrows* (panel *I*) indicate some very transient polypeptides, visible only at the first time point after an extreme heat shock

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Fig. 1. Fluorograms of two-dimensional gels of control (*A*) and heat shocked (*B*; 10 min at 45 °C) human lymphoblastoid cells (GM607)

In each case, the cells were labeled for 30 min with [^{35}S]methionine, beginning 1.75 h after the real or mock heat shock, and then solubilized. Various proteins increased by heat shock are *circled* (except for the enormous *HS:1* spot in panel *B*); the major proteins induced are labeled *HS:1–5* (short for "HShock:1–5"). Where appropriate, spots are also identified by a Master Spot Number (within the lymphoid cell system), e.g., *63* (*HS:1*). *M:1–3* are the mitochondrial proteins Mitcon:1–3. *SLS:1* is a short-lived species seen in such pulse-label experiments. *A* is actin, *T* is β-tubulin, μ and κ are the IgM heavy and light chains synthesized by this cell line, *L* is the B-chain of lactate dehydrogenase (EC 1.1.1.27), B_2M is β_2-microglobulin, and *nmTm* is non-muscle tropomyosin. Synthesis of most proteins is unaffected by the heat shock. Patterns in this and other illustrations of two-dimensional gels in this paper are oriented with the acid side to the left and the basic side to the right. Numbers on the vertical axis between the panels indicate molecular mass in thousands of daltons; the horizontal scale at the level of actin in Panel *A* indicates positions of charge standards

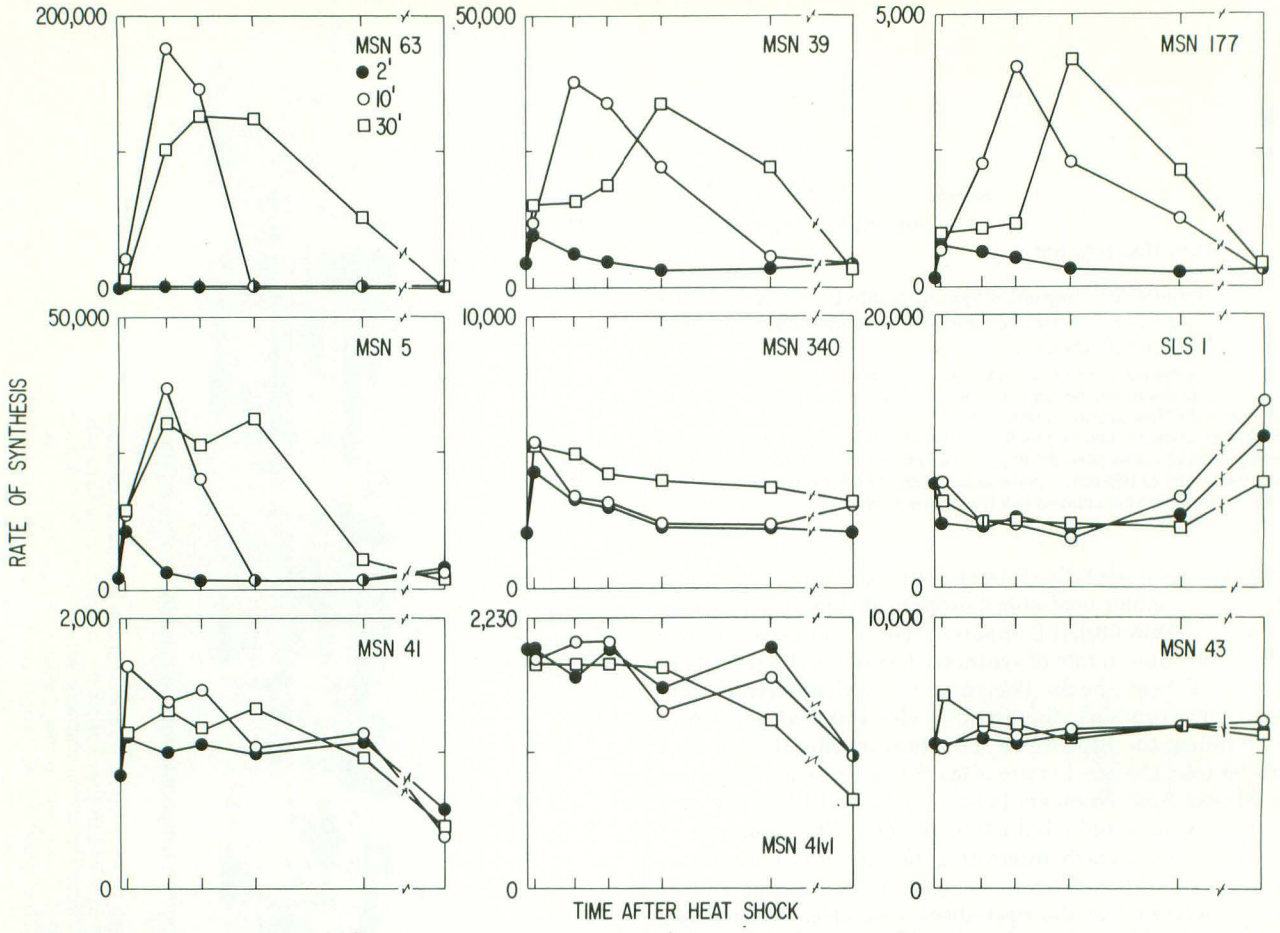

Fig. 4. Relative rates of synthesis of nine proteins throughout the time course shown in Figure 3 (*H–N*, 30-min heat shock) and analogous data from similar experiments in which the cells were heat shocked for 2 or 10 min

Quantitated by analyzing the fluorographs with the TYCHO computer system (*12*). MSN:63 is the major heat-shock protein (HShock:1), which is induced approximately 350-fold. MSN:39 (a mitochondrial protein, Mitcon:2), MSN:177, and MSN:5 are proteins induced reproducibly by heat shock, but not to the same degree as the classical heat-shock proteins. *MSN:43* (another mitochondrial protein, Mitcon:3), apparently unaffected by heat shock, was used together with Mitcon:1 to scale the data from different gels. Rates of synthesis are expressed in uniform, but arbitrary units; time points (from Fig. 3) are plotted linearly except for the last time (25 h)

nitely at the maximum reached after a single shock (Figure 5*G–J*). If each succeeding shock produced the same increase as the first, the relative amount of HShock:1 should increase substantially each day. Since it does not, there must be some mechanism limiting the accumulation of heat-shock proteins on an extended time scale.

Induction of the Heat-Shock Response by Other Agents and in Other Cells

Iodoacetamide, a compound that reacts quickly with protein sulfhydryl groups, induces the HShock set of proteins as efficiently as a heat shock (Figure 6*A–C*). Incorporation of amino acid analogs such as L-*threo*-α-amino-β-chlorobutyric acid (a valine analog, Figure 6*D,E*) or treatment with certain other agents, including cadmium, can also induce the response. However, neither high doses of butathionine sulfoximine [a potent inhibitor of γ-glutamylcysteine synthetase, (EC 6.3.2.2), necessary for cellular generation of glutathione; Figure 6*F*], nor inhibition of mitochondrial function (*11*), nor starvation of cells for glucose (data not shown) induces the HShock set. These results and others (*24*) make it unlikely that lack of intracellular glutathione (*3*, *25*), ATP (*2*), or glucose (*6*) is a major factor in inducing the response, but rather that the inactivation of proteins by the various inducers is responsible (*4*).

The major pattern of heat-shock-protein inductions in cultured skin fibroblasts is similar to that in lymphoid cells (Figure 6*G–H*), indicating that the form of response within a species need not be cell-type specific.

Of the normal mouse tissues examined, we found large amounts of a protein similar to HShock:1 only in the testis. However, the position of the testicular protein differs by about 0.6 charge unit from that of HShock:1 induced by heat shock in mouse spleen cells (Figure 7). Human testis contains a protein co-migrating with human lymphoid cell HShock:1 (Figure 8), but it is less abundant than the mouse testicular HShock:1 protein.

Observation of HShock:1 Induction in Fresh Human Leukocytes

When peripheral blood mononuclear cells from normal individuals are isolated by the standard Ficoll–Paque procedure and solubilized immediately for two-dimensional analysis, the cells contain low but detectable amounts of HShock:1 (Figure 9*A,B*). Cells from the blood of patients, sampled a day after surgery and similarly prepared (Figure 9*C,D*), show a much higher relative amount of the protein. Although such an increase may be at least partly the result of an increase in granulocyte contamination of mononuclear cells prepared from postsurgical samples (granulocytes normally contain relatively high amounts of HShock:1), these preliminary results suggest that major systemic shock (such as surgery) may induce HShock proteins in many cell types.

Purification of HShock:1 and Preparation of Specific Antibody

HShock:1 can be purified from extracts of heat-shocked human cells by chromatography on a column of DEAE-

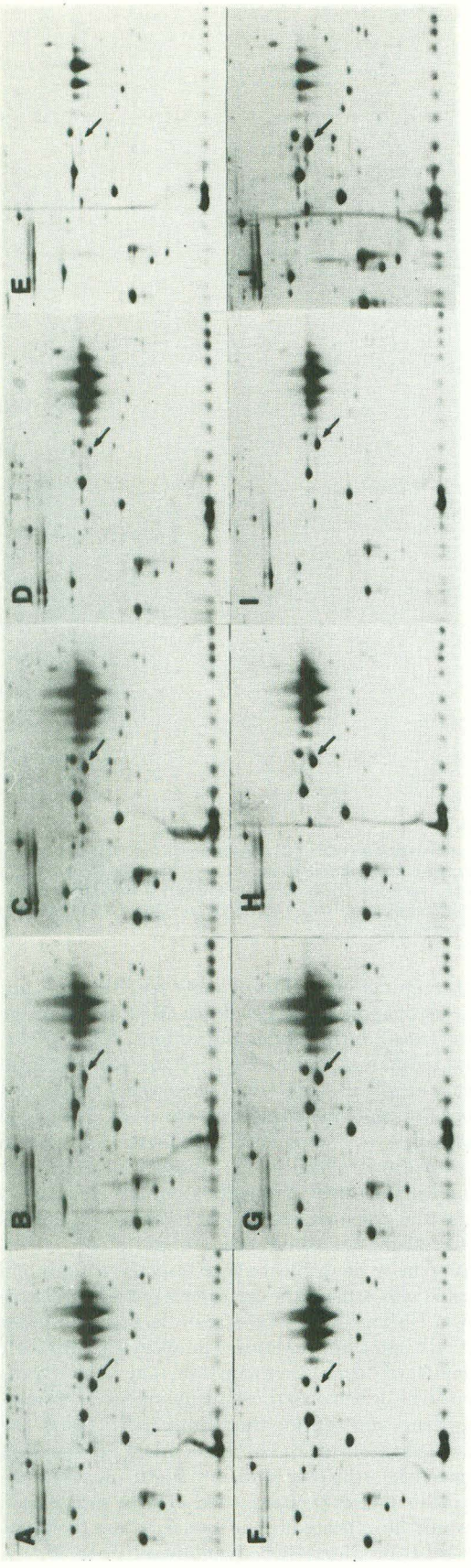

Fig. 5. Portions of Coomassie Blue-stained images of two-dimensional gels, showing the persistence of HShock:1 in human lymphoblastoid cells (GM607)

Panels *A–E* show the gradual decrease in HShock:1 (*arrow*) in cells one, two, three, four, or five days (respectively) after a heat shock. Panel *F* is an unshocked control. Panels *G–J* show the virtually constant proportion of HShock:1 maintained in cells heat shocked daily and solubilized for analysis 24 h after one, three, four, or five heat shocks, respectively. The horizontal rows of spots along the lower margin of each panel are internal standards (*19*)

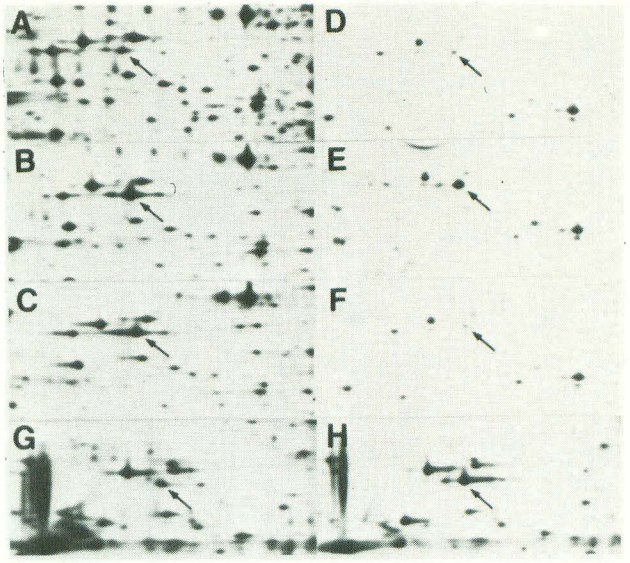

Fig. 6. Panels from autoradiographs showing increase in heat-shock protein under a variety of circumstances

(*A*) Control lymphoblastoid cells (GM607), (*B*) GM607 cells heat shocked for 20 minutes at 45 °C, (*C*) GM607 cells treated with 1 μmol of iodoacetamide throughout an 18 h labeling period, (*D*) human peripheral lymphocyte control, (*E*) lymphocytes treated with 20 mg of L-*threo*-α-amino-β-chlorobutyric acid per liter, (*F*) lymphocytes treated with 1 mmol of butathionine sulfoximine per liter, (*G*) human fibroblast control, (*H*) human fibroblasts heat shocked for 10 min at 45 °C. *Arrow* shows HShock:1

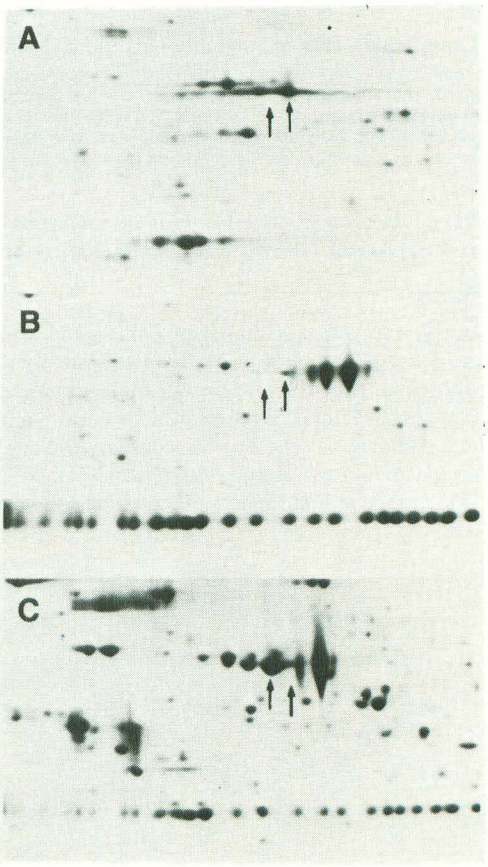

Fig. 7. Comparison of mouse spleen cell and presumed testicular heat-shock proteins

(*A*) autoradiograph of heat-shocked mouse spleen cells labeled with [^{35}S]-methionine; (*B*) stained image of the same gel as in *A*, showing position of mouse HShock:1 with respect to internal isoelectric-point standards; (*C*) stained image of a gel of mouse testicular tissue. The *right-hand arrow* indicates position of spleen cell HShock:1; the *left-hand arrow* indicates the position of the putative testicular HShock:1. The two proteins differ by about 0.6 charge unit in the isoelectric dimension, but have the same SDS-molecular mass

Sepharose CL-6B (Figure 10). When fractions of the eluate are analyzed by two-dimensional electrophoresis, HShock:1 is found to be eluted as a fairly pure species (>95%) in a single peak. Antibodies raised in rabbits to material so purified are specific to HShock:1 when used to stain a nitrocellulose transfer of the two-dimensional pattern of whole human

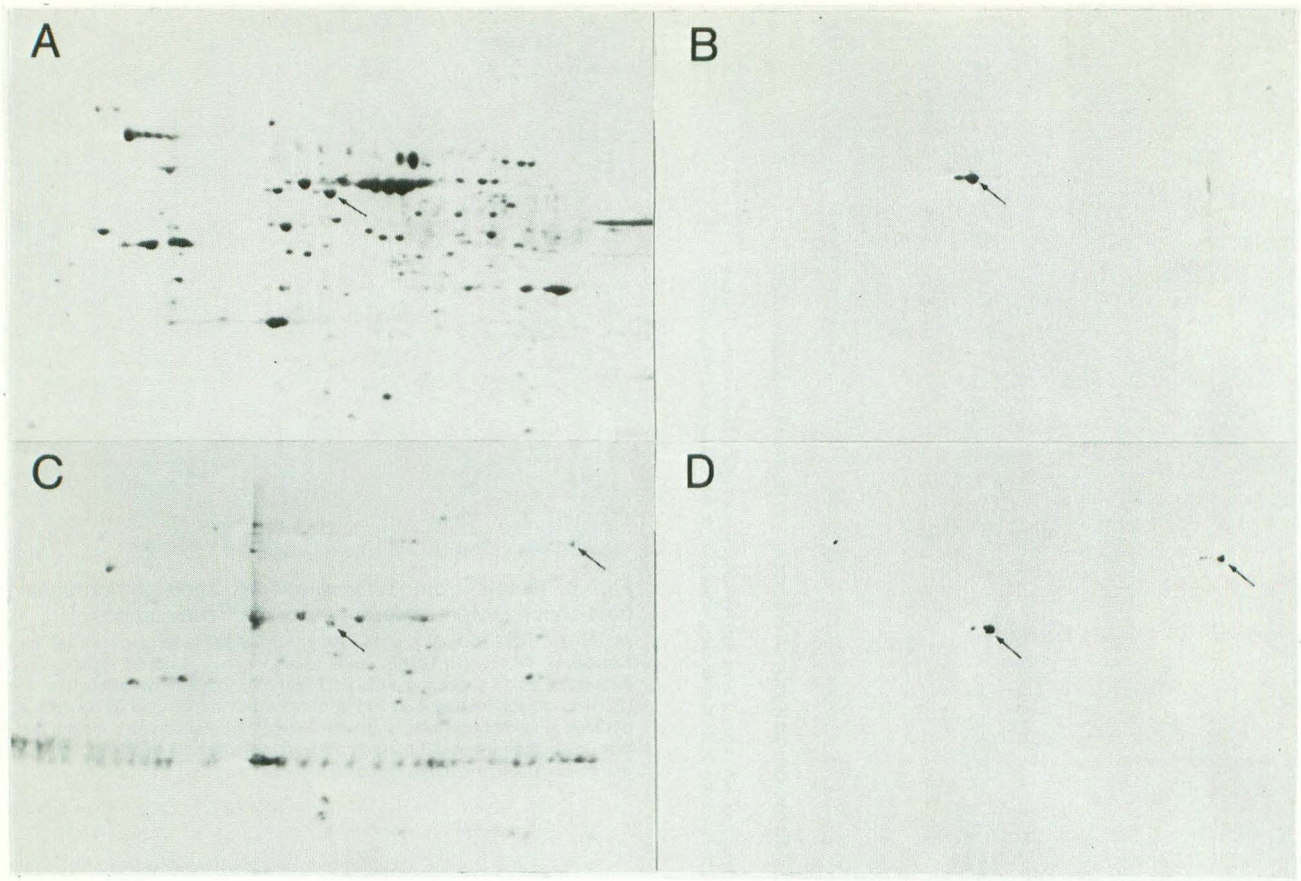

Fig. 8. Comparison of human lymphoblastoid and testicular HShock:1 proteins

(*A*) Autoradiograph of a nitrocellulose transfer of a two-dimensional gel of total lymphoblastoid cell protein labeled with [^{14}C]iodoacetamide (ref. *16*); (*B*) the same transfer stained with anti-HShock:1 antiserum by the anti-Ig peroxidase method; (*C*) autoradiograph of a nitrocellulose transfer of a two-dimensional gel of [^{14}C]-iodoacetamide-labeled human testicular tissue; (*D*) same transfer as *C*, stained with the anti-HShock:1 antibody. The position of HShock:1 is the same in human lymphoblastoid cells and testis. The antiserum reacts with one other testicular protein not present in lymphoblastoid cells. *Arrows* indicate HShock:1 and (in panels *C* and *D*) another protein in the testis (not present in lymphoblastoid cells) that reacts with anti-HShock:1 antibody

lymphoid cell proteins (Figure 11). Human testis contains one other protein recognized by the antibody (Figure 8).

Discussion

The fact that a system of similar proteins is induced by heat shock and other treatment in cells of a wide variety of animal species indicates that the response is a very basic aspect of higher biological systems. Even plants (*26*) and bacteria (*27*) show some response to temperature change. Although a wide range of treatments can induce the heat-shock proteins, an equally wide range of toxic agents do not. The cellular damage that causes the induction is thus of some specific type, not merely the result of approaching cell death. Metabolic damage (such as a decrease in intracellular glutathione, ATP, or glucose) appears unlikely to be the key, because experimental conditions specifically designed to provoke these conditions fail to produce the heat-shock response. However, damage to proteins that leads to a requirement for an increase in protein catabolism provides a plausible (*4*), though by no means proven, origin for the effect. Virtually all of the treatments that can cause the response are known to be capable of reacting with or causing the denaturation of proteins.

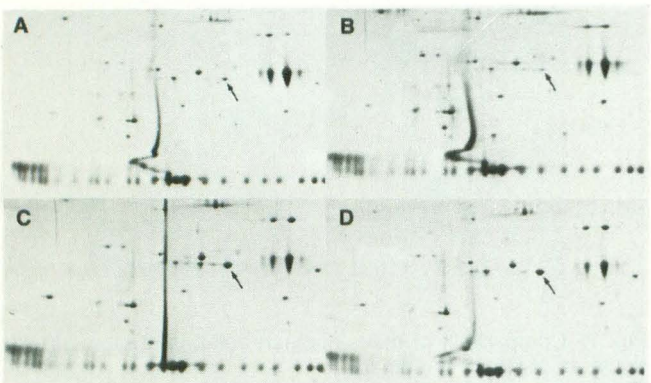

Fig. 9. HShock:1 in blood leukocytes (isolated by the Ficoll–Paque procedure)

(*A,B*) Coomassie Blue-stained two-dimensional patterns of leukocytes from healthy controls; (*C,D*) patterns of leukocytes taken from blood of two patients about 24 h after lengthy surgery. *Arrow* indicates HShock:1 protein

A relationship between the heat-shock response and protein catabolism could also explain the occurrence of higher relative amounts of the HShock:1 protein in granulocytes and testis than in other cells and tissues. Mature granulocytes, with their large complement of degradative enzymes, may be slowly digesting themselves, leading to the continuous production of denatured proteins and peptide fragments. In the testis, the necessity for spermatocytes to eliminate a large proportion of their cytoplasm in order to mature into sperm must require considerable catabolism of proteins—and a higher rate of such catabolism would be expected in the mouse, where the testis is maintained at approximately body temperature (~39 °C), as compared to man, where the testis is specifically situated for maintenance below 37 °C. This expectation is in agreement with the observation of very high relative amounts of an HShock:1-like protein in normal mouse testis as compared with the lesser (but still somewhat high) relative amounts in human testis. The peculiar testicular requirement for enhanced protein catabolism may thus be seen as mimicking the effect of heat shock, even at normal body temperature, and

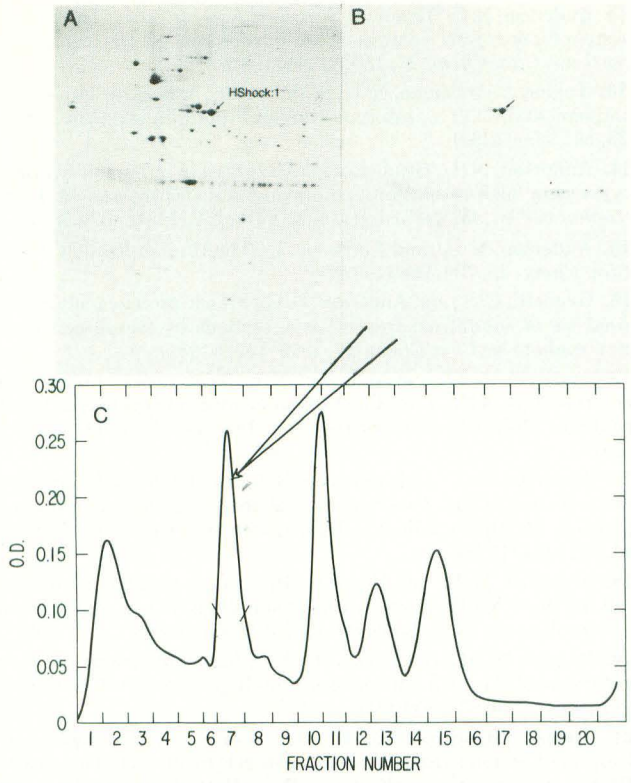

Fig. 10. Purification of human HShock:1 from heat-shocked lymphoblastoid cells

(*A*) Stained two-dimensional pattern of whole GM607 soluble proteins; (*B*) pattern of protein present in fraction 7 of the chromatographic eluate of soluble proteins; (*C*) optical density trace of eluate from the column described in *Materials and Methods; arrow* indicates peak of HShock:1. Horizontal rows of spots in panels *A* and *B* are internal isoelectric-point standards

therefore may account for the strong tendency in higher animals for the testis to be maintained below body temperature.

The mouse testicular HShock:1-like protein differs from the HShock:1 inducible by heat shock in spleen cells by about 0.6 of a charge unit. Their molecular masses appear to be identical. Such a fractional difference in charge suggests some difference in amino acid sequence, because most post-translation modifications that might account for tissue-specific differences involve alterations by integral charges. It therefore seems probable that the testicular and lymphocyte HShock:1 proteins are produced from different (though similar) genes, and perhaps constitute members of a multi-gene family. In man, the testicular, lymphocyte, and fibroblast HShock:1 proteins have precisely the same pI's (and sodium dodecyl sulfate molecular masses). This may indicate that, in man, all cell types employ the same single HShock:1 gene (in contrast to the rodents), or merely that these genes in humans all produce proteins of identical pI.

Quantitative analysis of the time course of the heat-shock response demonstrates that the effect is considerably more complex than was previously believed. While there is a set of a few proteins (HShock) that are strongly induced, there are also sets of proteins whose synthesis varies less strongly or even according to different response curves. Whether the latter effects are "important" parts of the heat-shock response, or just accidental alterations due to peculiar features of particular genes, remains unclear at this stage. Spot MSN:39 (a major mitochondrial protein, Mitcon:2 in ref. *11*) varies substantially, following a response rather similar to that of the HShock proteins, though with a total increase of only about 10-fold over normal values. In contrast, spot MSN:43, another major mitochondrial protein (Mitcon:3) seems to be largely unaffected by heat shock. An initial tentative identification of these two mitochondrial proteins as the β and α subunits of the F_1ATPase (*11*) seems less likely in view of the differences observed here in their regulation. Mitcon:3 (MSN:43) is perhaps more likely to be the α-subunit; Mitcon:1 and :3 seem equally unaffected by heat shock and maintain an apparently equal stoichiometry throughout the tissues (data not shown), while Mitcon:2 responds to heat shock and varies considerably from tissue to tissue.

Time-course analysis reveals also that the severity of the shock is related to the delay before maximum synthesis of HShock proteins is achieved. A 2-min shock at 45 °C produces only very small inductions, and those occur only at the 15-min time point. Ten- and 30-min shocks, however, produce maximum inductions of HShock:1 at approximately 2 h and 4–5 h, respectively. Thus greater damage results in slower response, as would be expected if the response depends for its execution on some cellular system that has itself been damaged.

The potential clinical importance of monitoring the induction of heat-shock proteins is obvious in some cases. Both fever and intentionally produced hyperthermia (as used in some cancer treatment regimens) exert stress on the tissues, which can reach unacceptable levels. The quantitative measurement of HShock proteins in plasma, leukocyte, or tissue samples, together with some knowledge of the time course of their induction, could afford a diagnostic measurement of the severity of stress. Such measurements could also be useful in

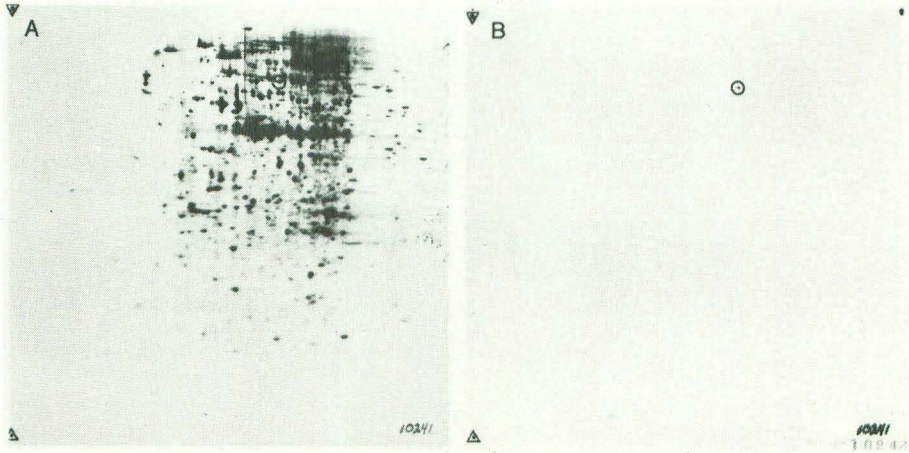

Fig. 11. Specificity of the anti-HShock:1 antiserum

(*A*) Autoradiogram of a nitrocellulose transfer of a two-dimensional pattern of [^{35}S]methionine-labeled human lymphoblastoid cells (GM607); (*B*) the same transfer stained with anti-HShock:1 antibody by the anti-Ig peroxidase technique. *Circles* enclose the HShock:1 spot

the evaluation or diagnosis of certain types of poisoning, including particularly that caused by heavy metals and sulfhydryl poisons. Now that antibodies specific to human HShock:1 have been obtained, we will be able to investigate these possibilities with a radioimmunoassay. In particular, the concentrations of HShock:1 in the plasma after induction of the heat-shock response in the whole animal must be quantitated. If successful, the development of a test based on HShock:1 will, to the best of our knowledge, represent the first clinical test devised to measure a change in cellular gene expression.

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