

6333

UNCLASSIFIED  
AS 7/2/00  
402905

AMRL-TDR-63-14

## THERMAL STRESS ON CELLULAR STRUCTURE AND FUNCTION

TECHNICAL DOCUMENTARY REPORT NO. AMRL-TDR-63-14

February 1963

Biomedical Laboratory  
6570th Aerospace Medical Research Laboratories  
Aerospace Medical Division  
Air Force Systems Command  
Wright-Patterson Air Force Base, Ohio

Contract Monitor: J. F. Hall, Jr.  
Project No. 7222, Task No. 722204

[Prepared under Contract No. AF 33(616)-8093  
by  
Ralph Buchsbaum  
Department of Biological Sciences  
University of Pittsburgh  
Pittsburgh 13, Pennsylvania]

## NOTICES

When US Government drawings, specifications, or other data are used for any purpose other than a definitely related government procurement operation, the government thereby incurs no responsibility nor any obligation whatsoever; and the fact that the government may have formulated, furnished, or in any way supplied the said drawings, specifications, or other data is not to be regarded by implication or otherwise, as in any manner licensing the holder or any other person or corporation, or conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

Qualified requesters may obtain copies from ASTIA. Orders will be expedited if placed through the librarian or other person designated to request documents from ASTIA.

Do not return this copy. Retain or destroy.

Stock quantities available at Office of Technical Services, Department of Commerce, \$0.75.

### Change of Address

Organizations receiving reports via the 6570th Aerospace Medical Research Laboratories automatic mailing lists should submit the addressograph plate stamp on the report envelope or refer to the code number when corresponding about change of address.

## **FOREWORD**

**The work described in this report was performed in the Laboratory for Experimental Cell Research, Department of Biological Services, University of Pittsburgh, Pennsylvania in partial fulfillment of Contract AF 33(616)-8093, Project No. 7222, "Biophysics of Flight," and Task No. 722204, "Thermal Stress in Extended Environments," administered by the 6570th Aerospace Medical Research Laboratories, Wright-Patterson Air Force Base, Ohio. Mr. John F. Hall, Jr., Chief, Biothermal Section, Biophysics Branch, Biomedical Laboratory, served as contract monitor. This investigation was initiated in April 1961 and concluded in July 1962.**

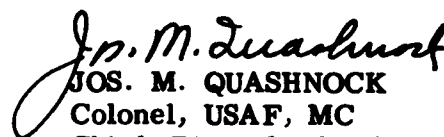
## ABSTRACT

Mouse kidney cells were grown on coverslips in a specially designed perfusion chamber which permitted continuous change of medium, microscopic examination and photography, and precise temperature measurement and control. Cells were exposed to normal body temperature for a time and then to an elevated temperature for a period of one to ten minutes. The temperature then was returned to normal. Observation of the cells revealed a standard pattern of response. Five stages of heat damage were distinguished in this cytosyndrome from normal to death of the cells. For each observation one hundred to one hundred fifty cells were each rated according to its appearance on a scale of five.

Cells appear generally normal up to 42° C. Studies were made at 47° , 50° , 53° , and 56° C. The time to reach any stage of damage is a factor of about 4.5 for each 3° C increase in heat-shock. The duration of exposure to elevated temperature was found not to be as important as the maximum temperature reached. A series of curves showing the distribution of cells in various stages of cell damage with temperature is presented.

## PUBLICATION REVIEW

This technical documentary report has been reviewed and is approved.

  
JOS. M. QUASHNOCK  
Colonel, USAF, MC  
Chief, Biomedical Laboratory

## I INTRODUCTION

This study is concerned with the response of mammalian cells in tissue culture to various periods of high temperatures. Such studies are important (1) to theoretical basic biology for the understanding of cellular function in relation to changes in the environment; and (2) to practical biology, especially to the Air Force, in the recovery of body cells after experiencing exposure to periods of high temperature to the point of burning.

Previous work in the field of time-temperature effects on cells in tissue culture has used the relative growth of the whole culture as an index of damage. At 42°C there are reported few observable changes in tissue-growth over that of tissues grown at 37°C. At 44°C some inhibition is seen, but changes are reversible.<sup>1,2</sup> It is interesting to note that the human heat-pain threshold is approximately 42°C to 43°C.<sup>7</sup> At 47°C definite lethal effects are seen.<sup>3,4</sup> Growth is retarded and cell shrinkage is observed, even after relatively short exposures. At higher temperatures, such as 50°C or 52°C, very short exposures can cause complete inhibition of growth, and often cell death. However, the pattern of heat-shock employed varies widely among investigators, perhaps because the rate of temperature increase and decrease was not controlled.

In this study, special apparatus has been developed<sup>5,6</sup> to allow more precise time-temperature programming as well as continuous observation of individual cells by phase-contrast photomicrography. In addition, and here reported for the first time, a semi-quantitative treatment of the morphological states of the individual cells has been used rather than the growth of the whole culture as an index of cellular response.

The implications of this present study of the heat response of the organelles of individual cells enables us to understand better the heat cytosyndrome (sequential pattern of reaction of cells to various degrees of heat damage). Heat-shocks of the order of magnitude described in this paper may occur in the cells of persons in extreme high-temperature environments or in the periphery of burns. Damage may occur without causing cell death, and the optimal time-temperature program to resist irreversible heat stress may be forecasted. Also it is possible that this information will lead to a method of study of the usefulness of various ways to prevent damage to heat-stressed cells. Or, it may provide a way to assess treatment of heat-stressed cells.

## II MATERIALS AND METHODS

Tissue was obtained aseptically from kidneys of a highly inbred strain DBA/2 mice ranging in age from 1-4 months.

### Cells

After the kidneys were taken from the animal, the capsules and renal vessels were removed, the remaining tissues minced with scissors and disaggregated with 0.5% trypsin in Hanks' balanced salt solution or in the GKN solution of Merchant, et al.<sup>8</sup> Cells and small aggregates thus obtained were centrifuged and resuspended in a medium consisting of 80% Hanks' balanced salt solution, 10% calf serum, and 10% Medium 199 plated onto 23 mm round 0-thickness coverslips in culture dishes containing the same medium, pH  $7.4 \pm 0.2$ . These preparations were incubated at 37.5°C for 2 to 6 days before being mounted into perfusion chambers.

### Perfusion Chamber-Heating Slide

The perfusion chamber used consists of several parts: (1) a 1" x 3" pyrex glass microscope slide having an electric current-conducting transparent coating across the bottom. (2) an aluminum frame attached by nontoxic epoxy resin glue to the slide, (Figure 1). This frame had ports allowing entry of perfusion lines and a thermocouple. The coverslip bearing the cells rested on top of the frame and was held in place by a metal ring screwed to the frame. A silicone rubber O-ring was placed within the frame and between the slide and the coverslip. This provided a chamber for the cells and medium such that the medium did not come into contact with any metal. A current was passed the length of the slide to produce heating of the fluid in the chamber.

The perfusion chamber, when assembled, was held firmly on the mechanical stage of the microscope by screws. Perfusion of medium was continuous at the rate of 1 ml/hour for the first 4 hours and 15 min/hour thereafter at the same rate. During the course of an experiment the microscope was kept at 36.5°C in a constant-temperature chamber, (Figure 2).

The temperature in the perfusion chamber was measured, controlled and recorded by a Leeds and Northrup Speedomox-H recorder and a series 60 Control Unit whose control current was amplified by a Fincor magnetic amplifier. The temperature measured by the thermocouple in the perfusion chamber was adjusted by the Control Unit to coincide with a control pointer.

The control pointer was moved upscale and downscale at an adjustable rate by a motor. The temperature in the chamber followed closely the position of the moving control pointer. The rate of temperature increase or decrease in the experiments reported here was five degrees per minute.

#### Experimental Technique

Tissue cultures were placed in the chamber for 2 hours at  $37.5^{\circ}\text{C}$  before an experiment. Then they were subjected to a heat shock as desired:  $47^{\circ}$ ,  $50^{\circ}$ ,  $53^{\circ}$ , or  $56^{\circ}$ . The duration of the elevated temperature varied from 1 minute to 10 minutes, (Figure 3). Then the temperature was returned to normal. Cells were observed and photographed on 35 mm film. From our observations we divided the process of cellular damage into five stages. For each experiment 100 to 150 cells were rated according to the scale at various time intervals after the heat-shock. The ratings of each cell population were then tabulated to determine the change in frequency of each stage with time under each experimental condition.

### III RESULTS AND CONCLUSIONS

Figure 4 shows the five stages in the degeneration of a single cell due to thermal shock. The five stages are:

#### Stage I. (Figure 4a)

The nucleus is large, round or slightly elliptical and agranular. The nucleoli are prominent and rounded in shape. The cytoplasm has few granules and many

filamentous mitochondria scattered throughout. The edge of the cell is transparent and smooth. Mitochondrial movement is continuous.

Stage II. (Figure 4b)

The nucleus may increase slightly in size (about 10%). Mitochondria and granules move toward the nucleus. Granularity may increase. The cell membrane may begin to shrink slowly.

Stage III. (Figure 4c)

The nucleus may be slightly deformed, or become more elliptical. The nucleoli appear to be more angular and of increased density. In the cytoplasm, long, filamentous mitochondria break up into shorter pieces, and the number of granules increases. Mobility of the mitochondria is radically reduced. Some small, clear vacuoles may form particularly at the less severe heat shocks. The cell membrane shrinks.

Stage IV. (Figure 4d)

The nucleus may be slightly granular, and smaller. The nuclear membrane often becomes indistinct, and apparent breaks have been observed. Long filamentous mitochondria are almost always absent, and there is a considerable increase in granularity and number of vacuoles. The cytoplasm is very shrunken; the cell membrane often exhibits sharply serrated edges. Vacuoles with a single granule inside showing brownian movement may be observed. The cell appears to freeze on time-lapse motion pictures.

Stage V. (Figure 4e)

The nucleus is small, dense, and granular. The nucleoli may be indistinct, or very dense, and reduced in size. The cytoplasm is completely filled with granules. A bled or blister-like pocket of clear fluid, may form hanging down from the coverslip about 100 microns on the average.

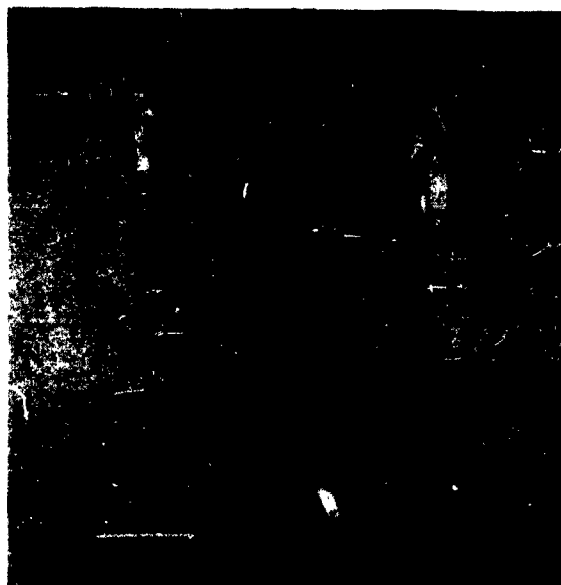


The cells which progress only to about Stage III may then recover. Different groups of cells on the coverslip may show different pictures. Separated only by a few hundred microns may be observed one group all dead and another with many Stage II or III cells. In rapidly growing tissue cultures the cells are likely to be more uniform than those used here. However, this study is concerned not with rapidly growing cells, but with surviving adult cells. Very high temperature shocks ( $60^{\circ}\text{C}+$ ) tended to fix the cell to the coverglass. The cytoplasm took on a slightly granular appearance within a few seconds, and no changes in morphology followed.

Duration of exposure to elevated temperature was not found to be as important as the maximum temperature. No difference in rate of damage was observed for  $53^{\circ}$  or  $56^{\circ}$  heat-shocks for periods of 1-10 minutes. But an exposure to  $50^{\circ}$  for one minute was significantly less harmful than  $50^{\circ}$  for 5 or 10 minutes. In no case of exposure to elevated temperature did a large proportion of cells survive, but reducing the exposure time did postpone death. Figure 5 shows a comparison between 10-minute and 1-minute heat-shocks. Note that the  $53^{\circ}$  and  $56^{\circ}$  curves are similar, but that the  $50^{\circ}$  curve is displaced to the right in the 1-minute heat-shock.

Time is required for differentiation of response. After 20 or 50 minutes, cultures exposed to a variety of heat-shocks look quite similar with very few Stage I cells, and many Stage II cells. By 250 minutes, however, those cells in cultures exposed to severe heat-shocks have died and those exposed to mild heat-shocks have "sickened" only slightly.

Figure 6 shows the results of a series of rating experiments. Some resistance to heat-shock is shown by a small proportion of the cells which had heat-shocks of  $47^{\circ}$  and  $50^{\circ}$ , but no cells survived  $53^{\circ}$  or  $56^{\circ}$  treatments. The rate of progression of stages of damage increases with increasing temperature. Data in



**Figure 1.** Perfusion chamber, showing from above downward: electric contacts, heating slide, aluminum frame, rubber o-ring, and top retaining ring. Width of heating slide 1 inch.



**Figure 2.** Perfusion chamber in place on microscope stage. Note thermocouple wire entering through 18-gauge hypodermic needle.

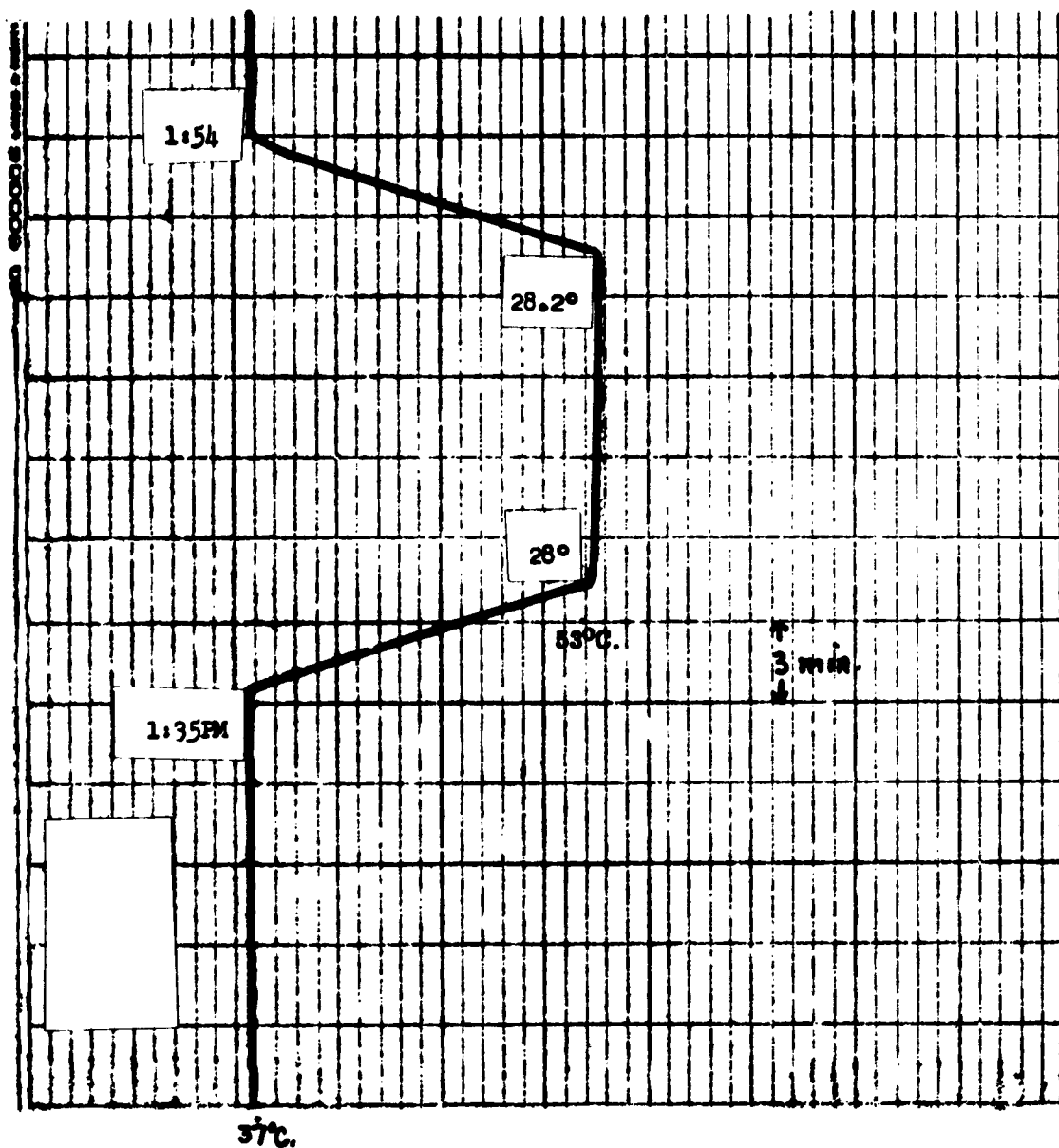
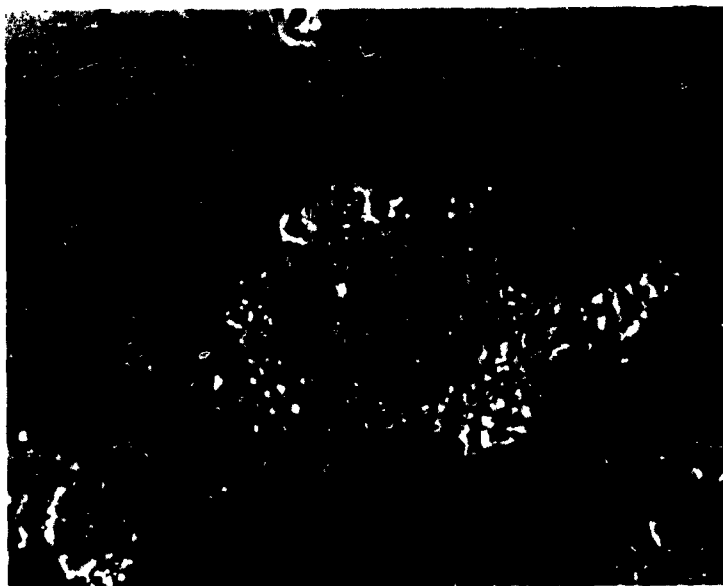


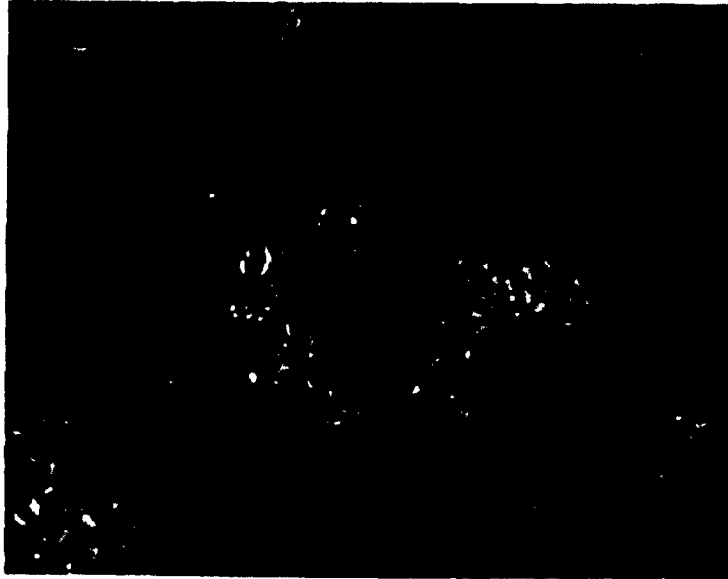
Figure 3. Typical experimental temperature record.



**Figure 4a. Heat cytosyndrome: Stage I.**



**Figure 4b. Heat cytosyndrome: Stage II.**



**Figure 4c. Heat cytosyndrome: Stage III.**



**Figure 4d. Heat cytosyndrome: Stage IV**



**Figure 4e. Heat cytosyndrome: Stage V.**

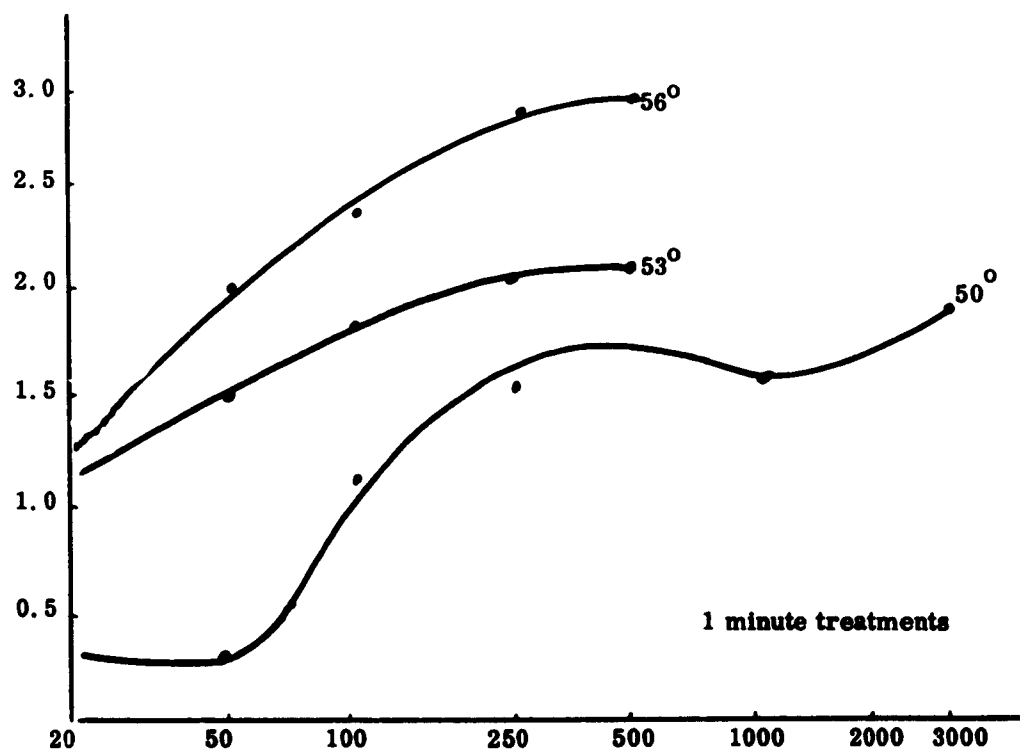
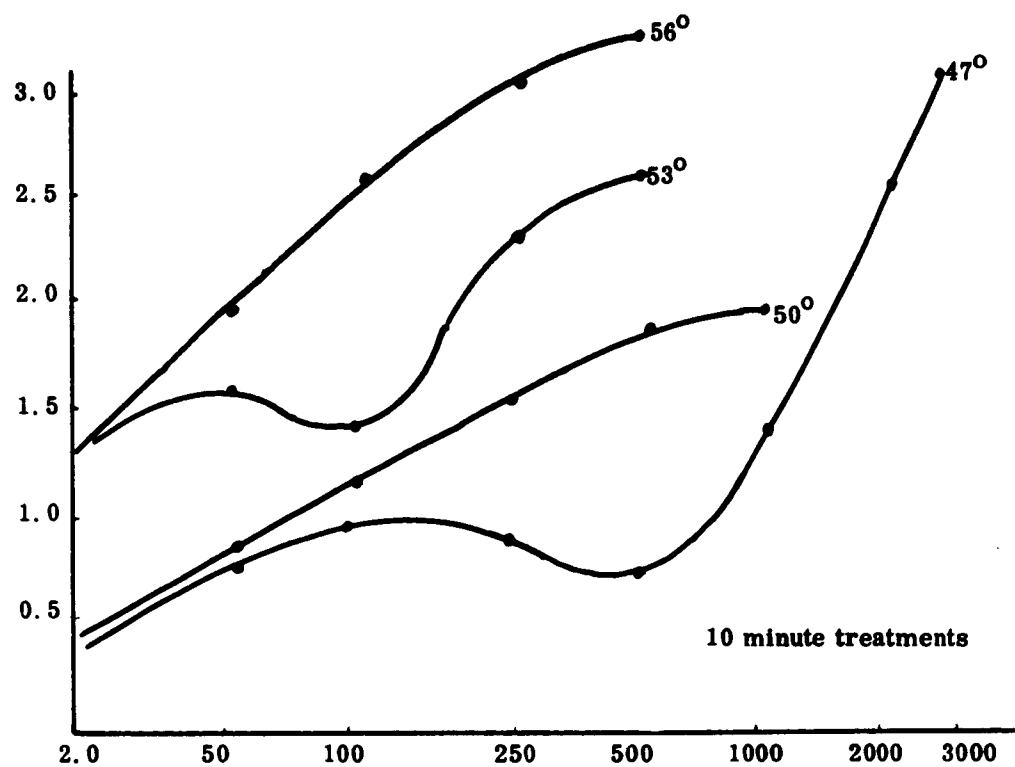


Figure 5. Average change in number of stages at various times after heat shocks at different temperatures.

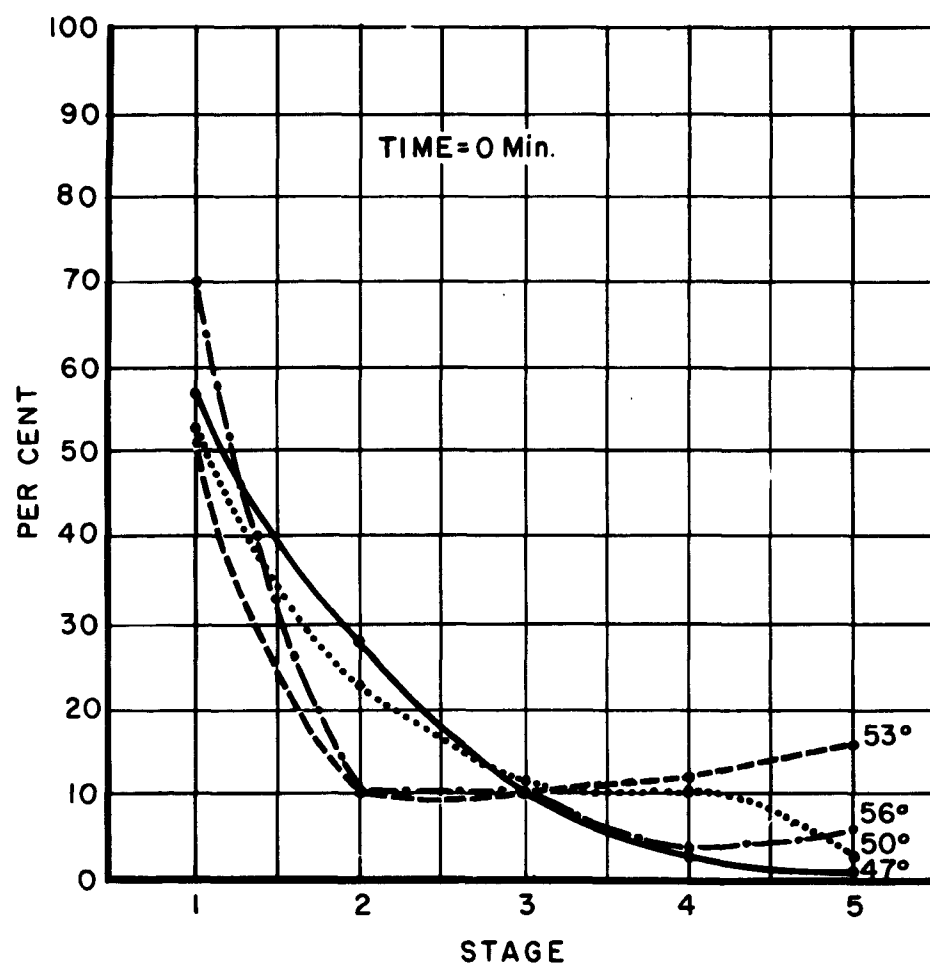


Figure 6a. Per cent of cells in each stage at the beginning of heat-shock experiments at four different temperatures.



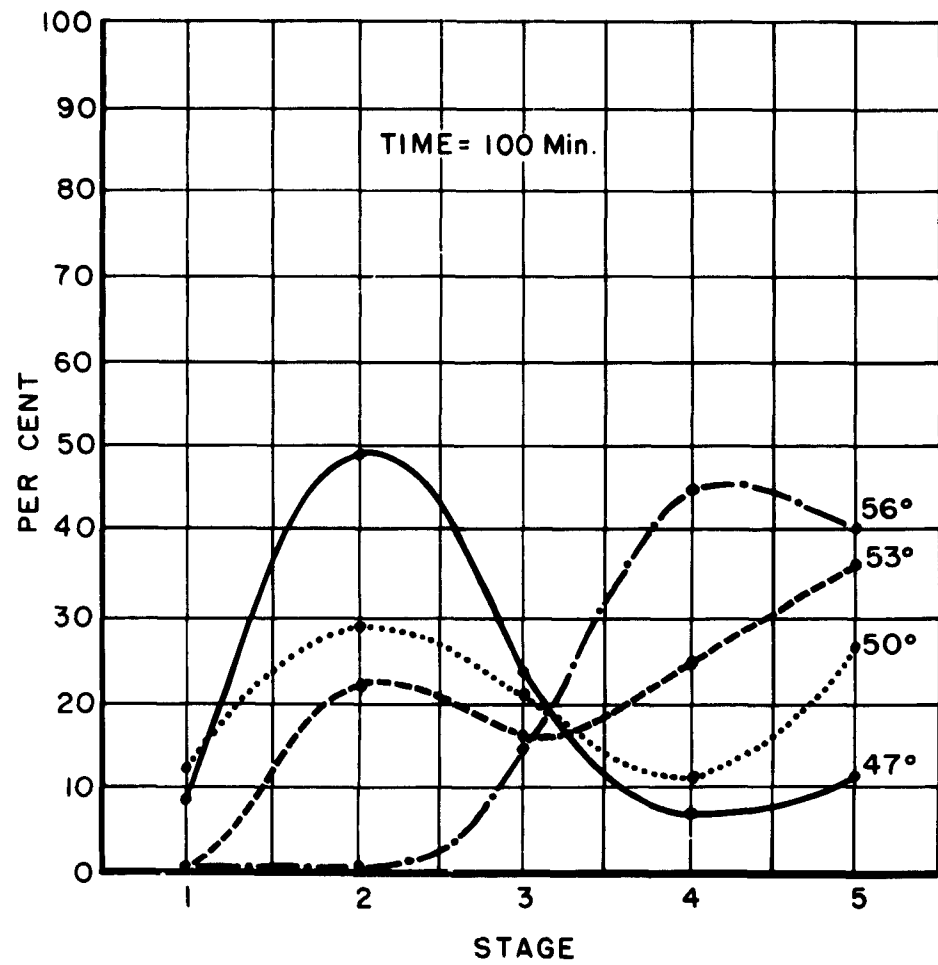


Figure 6b. Per cent of cells in each stage 100 minutes after a heat-shock experiment at four different temperatures.

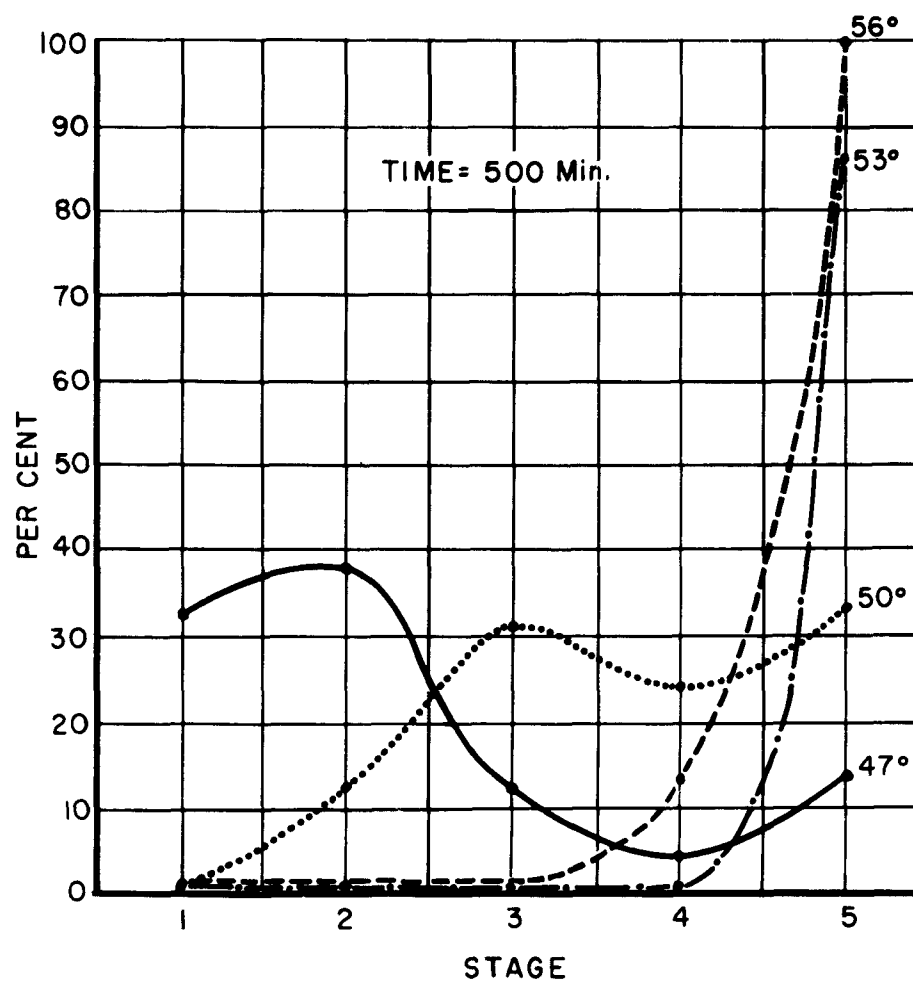


Figure 6c. Per cent of cells in each stage 500 minutes after a heat-shock experiment at four different temperatures.

Table I indicates that an increase of  $3^{\circ}\text{C}$  in the heat-shock accelerates the rate of appearance of damage by approximately a factor of 5.

TABLE I

<u>Heat Treatment For Ten Minutes</u>	<u>No. Minutes To Reach Different Degrees of Cell Damage</u>		
	<u>1.0 Stages</u>	<u>1.5 Stages</u>	<u>2.0 Stages</u>
$47^{\circ}$	100	1110	1510
$50^{\circ}$	71	225	1000
$53^{\circ}$	15	39	194
$56^{\circ}$	25	30	52

A summary of all experiments, for 10, 5 and 1 minute heat-shocks, shows that change in the number of minutes required to reach any stage of damage is a factor of 4.5 for each  $3^{\circ}\text{C}$  increase in heat shock. For example, if we increase the temperature of the heat-shock by  $3^{\circ}\text{C}$ , then the cells will take  $1/4.5$  times as long to reach any given value of damage.

Figure 6 also illustrates the variability of the rate of response of individual cells to heat shock. Variability is greater in heat shocks which are of less severity.

# REFERENCES

1. Frederic, J., "Recherches sur le comportement a temperature supranormale des nucleoles et du chondriome de fibroblastes cultives in vitro." Comptes Rendus des Seance de la Societe de Biologie et de ses Filiales 151 (1):188-190, 1957.
2. Firket, H., "Effet du chauffage de cultures de tissus a des temperatures supranormales sur la synthese de l'acide desoxyribonucleique." Comptes Rendus des Seance de la Societe de Biologie et de ses Filiales 151 (1):188-190, 1957.
3. Pincus, Gregory and Albert Fischer, "The growth and death of tissue cultures exposed to supranormal temperatures," Journal of Experimental Medicine, 54, (3):323-332, 1931.
4. Nakai, J., C.M. Pomerat, and T.G. Blocker, Jr. "Thermal stress and tissue culture VI. The effect of heat and time exposure on human epidermis and cells from the skin and leg muscle of chick embryos." Texas Reports on Biology and Medicine, 12 (3),:509-523, 1954.
5. Buchsbaum, Ralph and Joseph A. Kuntz, "The effects of certain stimulants and depressants on individual fibroblasts in a perfusion chamber." Annals of the New York Academy of Sciences, 58:1303-1310, 1954.
6. Buchsbaum, Ralph, Monte Buchsbaum and Joseph J. Migliore, "Temperature-time effects on mammalian cells in vitro." Abstracts of papers presented at the First Annual Meeting of the American Society for Cell Biology, November 2-4, 1961, page 22 of published abstracts.
7. Neisser, V., "Temperature thresholds for cutaneous pain," Journal of Applied Physiology, 14:368-372, 1959.
8. Merchant, D.J., Kahn, R.H., and Murphy, Jr., Wm. H. Handbook of Cell and Organ Culture, Burgess Pub. Co., Minneapolis, 1960.

9. Mixter, G. Jr., G.P. de Ihery, W.L. Derksen, and T.I. Monahan. "The influence of time on the death of HeLa cells at elevated temperature," Naval Material Laboratory, New York Naval Shipyard, Project 5046-16, Part 11, 1960.
10. Paul, John, Cell and Tissue Culture, Second Edition. Williams and Wilkins, Baltimore, 1960.

<p>Aerospace Medical Division 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio Rpt. No. AMRL-TDR-63-14. THERMAL STRESS ON CELLULAR STRUCTURE AND FUNCTION. Final report, Feb 63, iii + 17 pp. incl. illus., tables, 10 refs.      Unclassified report</p> <p>Mouse kidney cells were grown on coverslips in a specially designed perfusion chamber which permitted continuous change of medium, micro- scopic examination and photography, and precise temperature measurement and control. Cells were exposed to normal body temperature for a time and then to an elevated temperature for a period of one to ten minutes. The temperature then was returned to normal. Ob- servation of the cells revealed</p> <p>( over )</p>	<p>Aerospace Medical Division 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio Rpt. No. AMRL-TDR-63-14. THERMAL STRESS ON CELLULAR STRUCTURE AND FUNCTION. Final report, Feb 63, iii + 17 pp. incl. illus., tables, 10 refs.      Unclassified report</p> <p>Mouse kidney cells were grown on coverslips in a specially designed perfusion chamber which permitted continuous change of medium, micro- scopic examination and photography, and precise temperature measurement and control. Cells were exposed to normal body temperature for a time and then to an elevated temperature for a period of one to ten minutes. The temperature then was returned to normal. Ob- servation of the cells revealed</p> <p>( over )</p>	<p>UNCLASSIFIED</p> <ol style="list-style-type: none"> <li>1. Biothermal stress</li> <li>2. Histology</li> <li>3. Nuclei (biology)</li> <li>4. Cells (biology)</li> <li>5. Mice</li> </ol> <ol style="list-style-type: none"> <li>I. AFSC Project 7222, Task 722204</li> <li>II. Biomedical Laboratory</li> <li>III. Contract AF 33(616)-8093</li> <li>IV. University of Pittsburgh, Pittsburgh, Pennsylvania</li> <li>V. Ralph Buchsbaum</li> </ol> <p>UNCLASSIFIED</p>
<p>a standard pattern of response. Five stages of heat damage were distinguished in this cytosyndrome from normal to death of the cells. For each observation one hundred to one hundred fifty cells were each rated according to its appearance on a scale of five. Cells appear generally normal up to 42° C. Studies were made at 47°, 50°, 53°, and 56° C. The time to reach any stage of damage is a factor of about 4.5 for each 3° C increase in heat-shock. The duration of exposure to ele- vated temperature was found not to be as impor- tant as the maximum temperature reached. A series of curves showing the distribution of cells in various stages of cell damage with tempera- ture is presented.</p>	<p>a standard pattern of response. Five stages of heat damage were distinguished in this cytosyndrome from normal to death of the cells. For each observation one hundred to one hundred fifty cells were each rated according to its appearance on a scale of five. Cells appear generally normal up to 42° C. Studies were made at 47°, 50°, 53°, and 56° C. The time to reach any stage of damage is a factor of about 4.5 for each 3° C increase in heat-shock. The duration of exposure to ele- vated temperature was found not to be as impor- tant as the maximum temperature reached. A series of curves showing the distribution of cells in various stages of cell damage with tempera- ture is presented.</p>	<p>UNCLASSIFIED</p> <ol style="list-style-type: none"> <li>VI. In ASTIA collection</li> <li>VII. Aval fr OTS: \$0.75</li> </ol> <p>UNCLASSIFIED</p>