Results and discussion. Both the total cell and CFU-c concentrations increased after seeding the cultures, reaching a peak by week 4 at which time there were consistently higher concentrations in cultures with added substrates. Figure 1 shows CFU-c data for 36% of surface area coverage. The fall in the cell number after week 4 was associated with detachment of the adherent stroma. Figure 2 compares the CFU-c concentration at 4 weeks for all 3 groups. A consistent increase in CFU-c was noted which, in the case of eggshell membrane, appeared to be a function of the proportion of the surface area covered. A similar increase but of higher magnitude was observed with glass coverslips but here the concentration peaked with 36% coverage. The total cell count in the supernatant followed a similar pattern suggesting that the CFU-c concentration was a representative function of HSC in these studies.

Because similar cell numbers were used for the seeding of the cultures, the observed increases can only be attributed to enhanced support potential of the adherent stroma. This can come about because of either qualitative or quantitative difference in the adherent stromal layer or both. A quantitative increase in the density of the adherent stroma was observed on both added substrates using light and SEM. On glass coverslip, stromal cells covered both sides of the substrate in a higher density compared to plastic. The coating of both sides is possible because the coverslip is not tightly attached to the dish. By contrast, the eggshell membrane which was tightly attached to the dish was not coated on both sides, but the high cell density was evident. Structurally the glass surface appeared smooth on SEM, but the eggshell membrane appeared as a meshwork of branching fibrous structures, variable in thickness displaying multiple nodes from which emanated flat sheets which merged with the fibrous structures (fig. 3). This may provide a physical frame to support the adherence of stromal cells.

In glass surface, the higher density of adherent cells may simply be a pH effect[9]. Glass provides a relatively high pH of about 9. The cell surface, on the other hand, is negatively charged as demonstrated by strong staining with the positively-charged polycationic ferritin[4]. An electrostatic attraction may thus give a higher density of adherent cells on the glass, compared to plastic.

1 Supported by NIH grant No. 30142 to M. T.
2 Tavassoli, M., Exp. Hemat. *3* (1975) 213.
3 Dexter T.M., Allen, T.D., and Lajtha, L.G., J. Cell Physiol. *91* (1977) 335.
4 Dexter, T.M., and Testa, N.G., Meth. Cell Biol. *14* (1976) 387.
5 Tavassoli, M., and Takahashi, K., Am. J. Anat. *164* (1982) 91.
6 Tavassoli, M., Exp. Hemat. *10* (1982) 435.
7 Leighton, J., Mansukhani, S., and Estes, L.W., In Vitro *6* (1971) 251.
8 Leighton, J., Abaza, N., and Mansukhani, S., in: Tissue culture, methods and applications, p. 452. Eds P.F. Kruse and M.K. Patterson. Academic Press, New York 1973.
9 Bessis, M., Living blood cells and their ultrastructure, p. 146. Springer, Berlin 1973.

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Heat(40 °C)-induced polypeptides in human embryonic fibroblasts

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Summary. The synthesis of 3 polypeptides with molecular weights of 103-, 80- and 78-kdalton (K) was dramatically accelerated when the cultures of human embryonic fibroblasts were transferred from 37 to 40 °C. The induction of these polypeptides was not observed if actinomycin D was added to the cultures at the beginning of the rising of the temperature, indicating that this response may be mediated by increased transcription of their messenger RNA.

Recent studies have shown that a number of organisms induce the synthesis of a new set of polypeptides in response to elevated temperature[1-5]. The most extensively studied case is the heat-shock response of *Drosophila melanogaster* cells, and an analysis of the mechanisms has demonstrated that the heat-induced changes in protein synthesis may be mediated by a control acting at the level of translation as well as transcription[3]. We present here clear evidence to indicate that human cells are also capable of inducing preferential synthesis of a small number of polypeptides in response to elevated temperature.

Materials and methods. Human embryonic fibroblasts (HEF) were prepared from 3- to 5-month-old fetuses and grown at 37 °C in Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum, 100 units/ml of penicillin and 100 µg/ml of streptomycin. Two human tumor cell lines, Hela and Calu-1 cells, were also used in this study. The latter cell line of lung origin was kindly supplied by Dr Jørgen Fogh, Sloan-Kettering Institute for Cancer Research, New York. Polyacrylamide slab-gel electrophoresis (PAGE) was carried out by the method of Laemmli[6].

Samples were dissociated in 0.0625 M Tris-HCl (pH 6.8) containing 5% SDS, 2% 2-mercaptoethanol, 10% glycerol, and 0.001% bromophenol blue, followed by heating at 100 °C for 1 min. RNA polymerase (165-K, 155-K, 39-K), bovine serum albumin (68-K) and soybean trypsin inhibitor (21-K) were used as reference protein markers. After electrophoresis, the gels were fixed, dried and then exposed to Kodak Royal X-Omat films at −80 °C. Scanning of the film was carried out by a Shimazu dual-wavelength TLC scanner CS-910.

Results and discussion. As shown in figure 1, the incubation of HEF at 40 °C resulted in a change in the pattern of protein synthesis; the synthesis of polypeptides of sizes larger than 110-K was generally reduced whereas the synthesis of most polypeptides of sizes smaller than 80-K was slightly enhanced. The most striking change, however, was a dramatic increase in the synthesis of 3 polypeptides with molecular weights of 103-, 80- and 78-K. Essentially

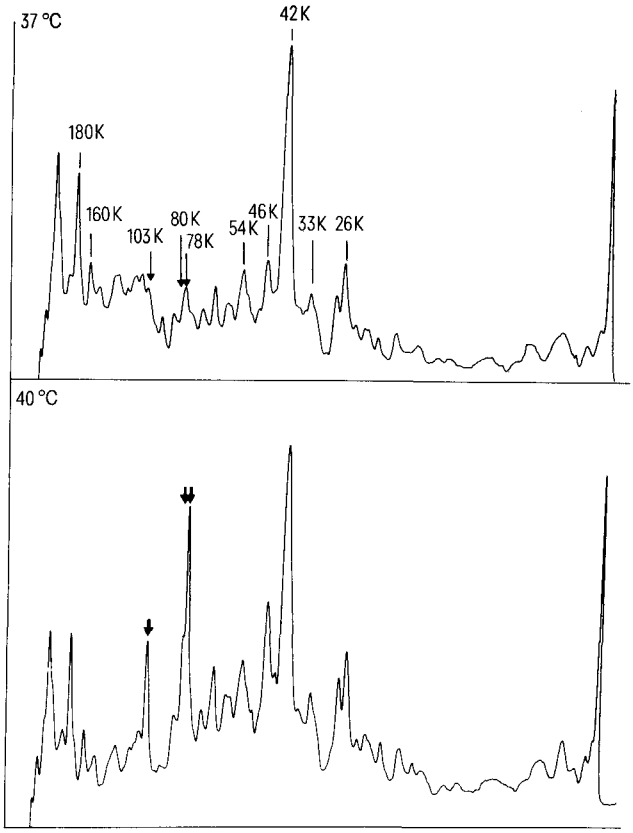

Figure 1. Effect of elevated temperature on the pattern of protein synthesis in HEF. *A* Autoradiogram of PAGE of ^{35}S-methionine-labeled HEF, *B* densitometer scanning of the autoradiogram. The 2nd subcultures of HEF were seeded onto 35 mm plastic dishes and incubated at 37 °C. After confluent cell growth, some of the cultures were transferred from 37 to 40 °C. After 5 h of incubation, cells were labeled with 5 µCi/ml of ^{35}S-methionine (sp. act.: 1250 Ci/mmole) for 1 h in methionine-free MEM at each temperature. The radioactive polypeptides were analyzed as described in 'Materials and methods'.

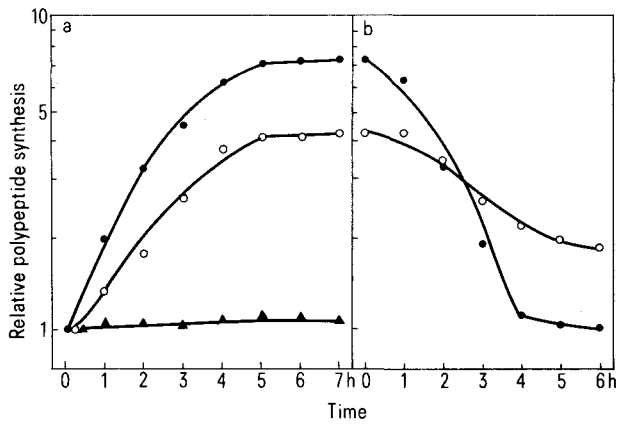

Figure 2. Time course of the induction and de-induction of 78- (●) and 103-K (○) polypeptides. *A* Confluent monolayers of HEF were labeled with 5 µCi/ml of ^{35}S-methionine for 1 h at various hours after shift-up to 40 °C. *B* Confluent monolayers of HEF were maintained for 7 h at 40 °C, transferred to 37 °C and then labeled with 5 µCi/ml of ^{35}S-methionine for 1 h at various hours after the temperature was lowered. Relative polypeptide synthesis expresses the ratio of the area under each polypeptide peak in the profile of densitometry from the temperature-shifted cultures to that in the profile from the control cultures maintained at 37 °C. The time course of the induction of 42-K polypeptide (▲) was shown as a control.

the same results were also obtained with other 3 cell lines of HEF derived from different fetuses although there were minor variations among cultures in the extent of the induction of these polypeptides. When HEF were transferred from 37 to 40 °C, the rate of synthesis of 78- and 103-K polypeptides increased gradually and reached a maximum level at 5 h after shift-up (fig. 2, A). The enhanced synthesis persisted for at least 4 h even if the temperature was lowered from 40 to 37 °C. The synthesis of the 78-K species was decreased at a faster rate than that of the 103-K species (fig. 2, B). Such enhancement of the synthesis of 78- and 103-K polypeptides was not observed when actinomycin D (1 µg/ml) was added to the cultures at the beginning of the rise in temperature. Pulse-chase experiments demonstrated that there was no difference between the degradating rates of these polypeptides at 37 °C and at 40 °C (data not shown). These results suggest that the de novo synthesis of messenger RNAs was required for the selective induction of 78- and 103-K polypeptides. As shown in figure 2, A, the synthesis of 42-K polypeptide which was thought to be actin, a major cellular component[7,8], was neither enhanced nor suppressed by temperature shift-up from 37 to 40 °C. A similar set of polypeptides were preferentially synthesized at 40 °C in human cancer cell lines, Hela and Calu-1 cells. The enhancement, however, was not as prominent as that observed in embryonic fibroblasts. In the cancer cells, the synthesis of 103-K polypeptide was present at a relatively high level even at 37 °C (data not shown).

The present study demonstrates that an elevation of temperature by only 3 °C over the normal physiological temperature resulted in a strong induction of a set of polypeptides of human embryonic cells without a concomitant deceleration of synthesis of the other polypeptides. Since HEF can grow well and can be maintained for a long time at 40 °C[9,10], these induced polypeptides may play a positive role in the survival of cells under conditions outside the physiological range. Similar changes in the pattern of protein synthesis may occur at the organismal level when the human body temperature rises to as high as 40 °C because of severe viral or bacterial infections. Although the function of the heat-induced polypeptides is unknown, these polypeptides might be involved in various diseases causing high fever such as infections and metabolic disorders.

1 Kelley, P.M., and Schlesinger, M.J., Cell *15* (1978) 1277.
2 Ashburner, M., and Bonner, J.J., Cell *17* (1979) 241.
3 Stori, R.V., Scott, M.P., Rich, A., and Pardue, M.L., Cell *22* (1980) 825.
4 Levinson, W., Oppermann, H., and Jackson, J., Biochim. biophys. Acta *606* (1980) 170.
5 Key, J.L., Lin, C.Y., and Chen, Y.M., Proc. natl Acad. Sci. USA *78* (1981) 3526.
6 Laemmli, U.K., Nature *227* (1970) 680.
7 Pollard, T.D., and Weihing, R.R., CRC Crit. Rev. Biochem. *2* (1974) 1.
8 Brag, D., and Thomas, C., Biochem. J. *147* (1975) 221.
9 Thompson, K.V.A., and Holliday, R., Exp. Cell Res. *80* (1973) 354.
10 Nishiyama, Y., and Rapp, F., J. gen. Virol. *52* (1981) 113.

0014-4754/83/040412-02$1.50+0.20/0