PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 164, 121–127 (1980)

Selective Increases in the Activities of Rat Liver Polysomes following Burn Trauma[1] (40834)

PAMELA M. HURLEY AND DAVID IRWIN[2]

Cell Biology Unit, Shriners Burns Institute, Department of Surgery, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

Severe trauma causes profound changes in tissue protein metabolism (1, 2,) that are particularly complex in the liver. Proteolysis for energy production takes place that does not respond to infusions of glucose, and hypoalbuminemia occurs despite evidence of increased overall protein synthesis rates. Studies of protein synthesis in hepatic tissue during dietary protein deficiency indicate that synthesis of certain proteins is elevated whilst that of others is depressed (3, 4). V. M. Pain *et al.* (5) suggest that selective alterations in rates of hepatic protein synthesis may occur via the availability of specific mRNAs. Another possible mechanism for regulation could be at the level of translation of mRNAs, such as that found in the differential translation rates of α- and β-globin (6–8). Although there is 40% more α-globin mRNA than β-globin mRNA, production of the two proteins is exactly balanced because of a higher rate of initiation of translation on the part of β-messenger. Regulation is presumably mediated by initiation factors or other proteins rather than by any differences between the two mRNAs. In the study described here, we have examined the effects of severe trauma (large, full thickness skin burn) on protein synthesis by hepatic polysomes both *in vivo* and *in vitro*.

Materials and methods. Preparation of polysomes labeled in vivo. Male Sprague–Dawley rats weighing 200–250 g (Charles River Laboratories, Wilmington, Mass.) were maintained on a diet of rat chow and water ad libitum until 18 hr before sacrifice. They were then administered 40 mg/kg pentobarbitol i.p. and either given a 20–30% full thickness scald burn or a sham burn using a template on the animal's back and revived with an injection of saline i.p. Groups of three animals were sacrificed at 1-, 5-, and 12-day postburn. Each animal was starved overnight and administered 1 μCi/g [^{3}H]leucine (New England Nuclear Corp., Boston, Mass.) s.c. 1 hr prior to sacrifice. Since ribosome half-lives are from 4 to 6 days, this should predominantly label growing nascent polypeptide chains plus newly synthesized and released proteins. Livers were removed after cervical dislocation, pooled, and then placed on ice in 1 g/4 ml RSB buffer (10 m*M* Tris, 10 m*M* NaCl, 1.5 m*M* $MgCl_2$, pH 7.4, with HCl). All further procedures were carried out at 4°C. The tissues were homogenized in a Dounce homogenizer (eight strokes of the loose pestle, four strokes of the tight pestle) and centrifuged at 12,100 *g* for 10 min in a Sorvall RC2-B centrifuge. One milliliter of supernatant was precipitated with excess 5-sulfosalicylic acid to remove proteins. The acid-soluble fraction was analyzed on a Beckman amino acid analyzer (Beckman Instruments, Inc., Palo Alto, Calif.) to determine the leucine concentration. Radioactivity was measured using TX-8 (1 vol Triton X-100, 2 vol xylene, 8 g/liter Omnifluor (New England Nuclear Corp., Boston, Mass.)) as the scintillation fluid: counting efficiencies were 35%. The ratios of leucine cpm/nmol leucine were used as indicators of leucine pool specific activities.

The remaining supernatant was divided into 1-ml aliquots and 50 μl of a 10% solution of Nonidet P-40 was added to each. The 1-ml aliquots were each layered on top of 15–40% linear sucrose gradients (sucrose buffer: 100 m*M* NaCl, 10 m*M* $MgCl_2$, 10 m*M* Tris, pH 7.4, with HCl) and centrifuged for 3 hr at 90,000 *g* in a Beckman SW27 ultracentrifuge rotor. The sucrose gradients were fractionated whilst moni-

[1] Supported by NIH Grants GM 21700, GM 07035, and CA 20634.

[2] Present Address: Department of Biochemistry, George Washington University Medical Center, 2300 Eye Street, N.W., Washington, D.C. 20037.

0037-9727/80/060121-07$01.00/0

toring the A_{260} using a spectrophotometer flow cell (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). The proteins of each fraction were precipitated with an equal volume of 20% trichloroacetic acid (TCA), filtered and rinsed on GF/A Whatman glass fiber disks (Fisher Scientific Co., Fairlawn, N.J.) with cold 10% TCA, 5% TCA, and ethanol, and the radioactivity determined. The specific activity of each fraction was derived by dividing the counts per minute value by the absorbance of that fraction at 260 nm. The polysome sizes were extrapolated for each fraction by reference to the single ribosome peak at 80 S.

Preparation of membrane bound and free polysomes. Preparation of cell sap and liver polysomes was according to the procedure of Ramsey and Steele (9). Groups of burned and sham-burned animals were anesthetized with ether and prepared for liver perfusion under ether anesthesia. Livers were perfused via the portal vein with 40 ml ice-cold 0.25 *M* sucrose in 1 m*M* $MgCl_2$ solution. Each liver was homogenized in polysome buffer containing 250 m*M* sucrose (3 ml/g liver) with 10 strokes of a motor-driven pestle (Thomas Co., Philadelphia, Pa.), centrifuged at 740 *g* in a SW27 Beckman rotor for 2 min, and then centrifuged at 131,000 *g* for 12 min. The supernatant was decanted and saved for analysis of free polysomes.

Each pellet was resuspended in liver cell sap (9), using a motor-driven Teflon pestle and 10% Triton X-100 added to a final concentration of 1%. The homogenate was centrifuged at 1470 *g* for 5 min in a RC2-B centrifuge and 13% (w/w) sodium deoxycholate was added to a final concentration of 1.3% in order to release membrane-bound polysomes.

Three-milliliter aliquots of free and membrane-bound polysomes were layered separately over discontinuous sucrose gradients (3 ml each of 1.3 and 2.0 *M* sucrose in cell sap, polysome buffer, and 250 m*M* KCl) and centrifuged at 174,000 *g* for 20 hr in a Type 65 Beckman rotor. The yield of free and membrane-bound polysomes was approximately 8 OD and 16 OD units/g liver, respectively, for both burn and sham animals.

Measurement of polysome size distribution. Pellets of membrane-bound and free polysomes were resuspended in 700 and 200 μl respectively, of polysome buffer and 25 μl of each were removed for cell-free protein synthesis. The remainder was taken up in a total of 2 ml polysome buffer, layered over 15–27.8% (w/w) linear sucrose gradients containing 10 m*M* Hepes (pH 7.4), 75 m*M* KCl, 5 m*M* $MgCl_2$, and 0.5 m*M* EDTA and centrifuged at 131,000 *g* for 105 min in a SW27 rotor. The absorbance profile was read at 260 nm and the polysome fractions were divided into five size groups. Each of the five fractions was centrifuged overnight at 174,000 *g* and the resulting pellets were resuspended in buffer and protein synthesis activities were determined *in vitro*.

Measurement of cell-free protein synthesis. A rabbit reticulocyte lysate protein synthesizing system in which endogenous mRNA is inactivated by micrococcal nuclease was formed by the method of Pelham and Jackson (10–12). Protein synthesis was measured using [^{35}S]methionine (25 μCi/ml, New England Nuclear Corp., Boston, Mass.) at a dilution of 1:20. The test polysomal fraction was added to each tube and assayed as previously described (10). The 5-μl aliquots of products were TCA precipitated, filtered through Millipore filters (Millipore, Bedford, Mass.), and washed twice with 5% TCA, and once with 70% ethanol. The filters were dissolved in 5 ml Brays solution (New England Nuclear Corp., Boston, Mass.) and radioactivity was determined in a scintillation counter (counting efficiencies of 60–70%). The remaining lysate fractions were made 0.4% in SDS, 1% in 2-mercaptoethanol, and heated at 70°C for 15 min. Hemin was removed by centrifugation at 8000 *g* for 2 min. Proteins were resolved in 10% polyacrylamide gels (13), stained with Coomassie blue, and destained overnight in a 10% acetic acid–propanol solution.

The statistical significance of the differences between groups was assessed by Student's *t* test.

Autoradiography of SDS–polyacrylamide gels. The SDS–polyacrylamide gel was placed in DMSO and shaken for 1 hr at 30°C. The DMSO solution was

changed and the procedure was repeated for 1 hr. The gel was then placed in 22% Omnifluor in DMSO and shaken for 3 hr at room temperature. The Omnifluor–DMSO was removed and the gel was washed with running water for 1 hr and then dried on a slab-gel dryer (Bio-Rad, Rockville Center, N.Y.). The dried gel was placed with X-OMAT RP film (Kodak, Rochester, N.Y.) in a black folder and placed in the freezer for 4 to 6 weeks. The film was developed for 4 min with developing fluids A and B (E. M. Parker Co., Brookline, Mass.), washed in a running water bath, and then fixed 4–5 min in fixer (940 ml rapid fixer A and 106.4 ml hardener B to 1 gallon with water). The final developed film was rinsed for 10 min with water and suspended to air dry.

Results. Incorporation of [^3H]Leucine in vivo into nascent polypeptide chains. Figure 1a shows the absorbance profiles (A_{260}) and [^{3}H]leucine incorporation patterns (cpm) versus fraction number of representative rat liver polysomes from 1-, 5-, and 12-day postburn, and sham-burn animals. At 1- and 5-day postburn, the mean peak polysome size was increased from 9 monomers (sham) to 10–12 monomers. By 12 days the peak size had reverted to sham values. The ratio of monosomes to polysomes (mean ± SD) was approximately 0.1 ± 0.01 for the sham-burn animals and 0.06 ± 0.01, 0.08 ± 0.01, and 0.12 ± 0.01 for the 1-, 5-, and 12-day postburn animals, respectively. However, burn trauma did not affect the total yield of polyribosomal material. The specific activities of the polysomes (cpm/A_{260}) are shown in Fig. 1b.

Figure 2 shows the specific activities for five different polysomes size groups. The largest increases in specific activities were observed at 1-day postburn (500–930 cpm/A_{260}). At 5 days, although peak polysomes remained at an increased size, the specific activities had returned to sham values (300–350 cpm/A_{260}). By 12-day postburn, specific activities were depressed by approximately 15%. Measurements of leucine pool size in both burn and sham animals indicated that these activity changes were not due to changes in specific activity of leucine pools in the liver. The leucine concentrations for 1- and 5-day burns were 199 and 281 nmol/g wet wt of liver, respectively. The corresponding 1- and 5-day sham values were 223 and 230 nmol/g wet wt, respectively. Leucine specific activities for 1- and 5-day burns were 2.12×10^3 and 1.82×10^3 cpm/nmol, respectively: 1- and 5-day sham values were 1.49×10^3 and 1.91×10^3 cpm/nmol, respectively. The greatest increases in specific activities of the polysome size groups occurred in the smallest and largest polysomes. Increases over sham values ranged from 70 (9–11 monomers) to 170% (2–4 monomers).

Incorporation of [^{35}S]methionine into proteins by polysomes in vitro. Figure 3 compares the stimulation/g liver (mean ± SD) over a blank assay of 1- and 5-day postburn liver polysomes with sham polysomes (free and membrane bound) using the rabbit reticulocyte lysate cell-free system. The mean stimulation with free polysomes isolated from rat livers 1 day following burn injury was greater than the corresponding sham values ($P < 0.05$). However, at 5 days following burn injury, the stimulation values from the burned animals had returned to sham levels. At 1-day postburn, both sham and burn polysomes had a higher mean stimulation than polysomes isolated from normal rats. These values were not statistically different from the normal levels at 5-day postburn ($P < 0.9$).

Autoradiography of electrophoresed reticulocyte lysate products (Fig. 4) showed that although the free and membrane bound lysate products contained a similar SDS electrophoretic pattern of stainable proteins (Fig. 4a), the free polysome lysate products contained many more electrophoretic bands of newly synthesized proteins that had incorporated [^{35}S]methionine (Fig. 4b). However, the stimulation of the membrane-bound polysomes in both 1-day burn and sham animals was slightly greater than the mean free polysome values, though not statistically different because of biological variation ($P = 0.6$). There is some indication that the membrane-bound-polysome-synthesized products may not enter the 10% polyacrylamide gel as easily as the free polysome products.

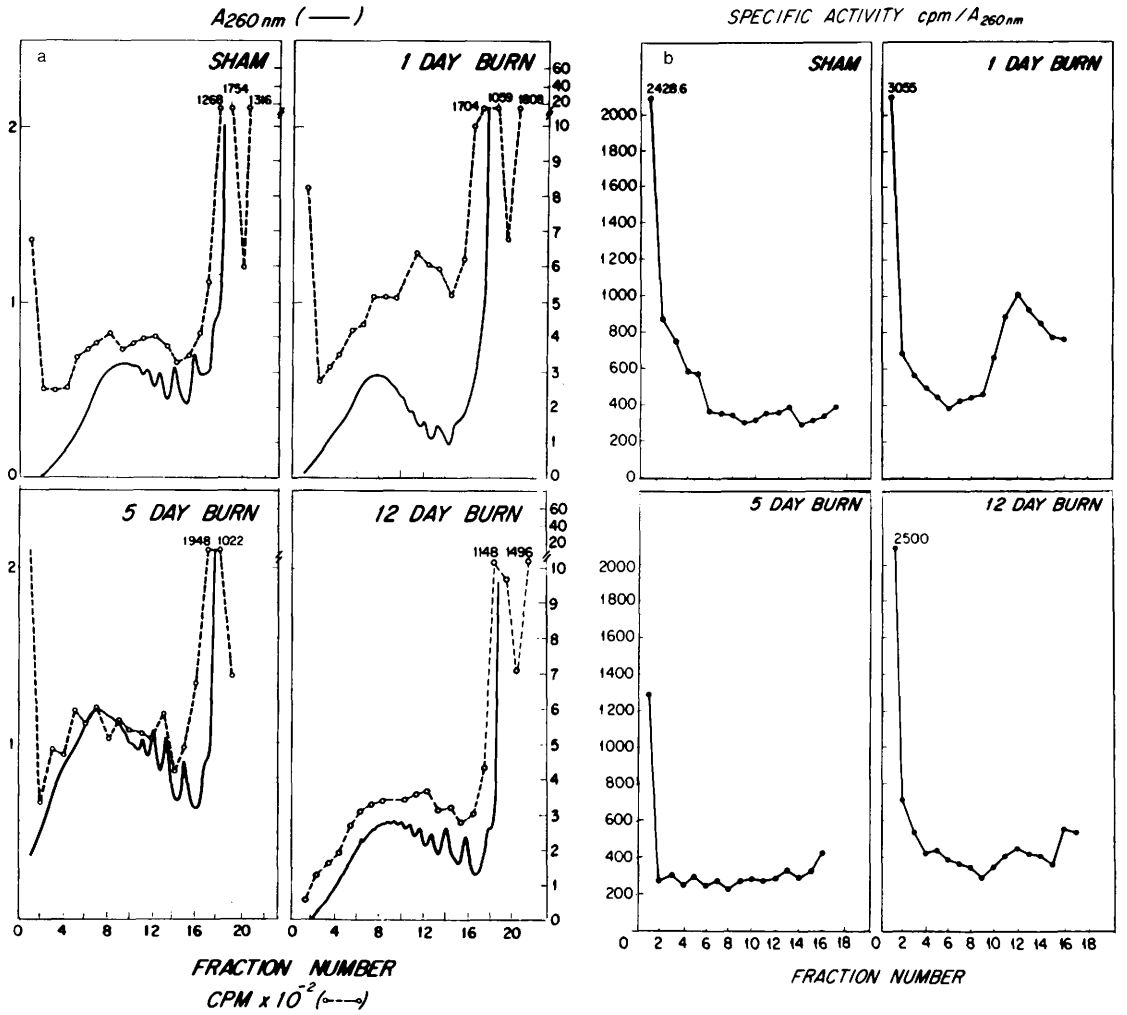

FIG. 1. (a) Incorporation of [^{3}H]leucine into rat liver polysomes. Rats in groups of three were injected s.c. with [^{3}H]leucine (1 μCi/g body weight). One hour later, livers were pooled and polysomes were isolated and fractionated on sucrose gradients as described under Materials and Methods. The absorbance at 260 nm was measured for each fraction and the radioactivity of TCA-precipitable material was determined. Polysomes were isolated at 1, 5, and 12 days after a full-thickness skin burn and from animals that received a sham burn. Absorbance at 260 nm (——); radioactivity, cpm $\times$ 10^{-2} (O--O). (b) Specific activity of polysome fractions. Specific activities (radioactivity/absorbance) were calculated from the polysome profiles in (a) for each animal group. cpm/A_{260} (●——●).

In an attempt to determine whether any particular polysomal size class was responsible for the increase in activity during burn injury, we divided the total membrane-bound and free polysomes into five fractions by size and tested each fraction for protein synthesizing capacity in the reticulocyte system. Figure 5 compares the mean stimulation/g liver of the five different polysome size classes from 1-day postburn animals and their corresponding shams. The most active class was the free 195- to 280-S size class (F3). Again, the sham and burns were greater than normals, but not to a significant degree ($P > 0.2$) except in several of the membrane-bound fractions. The stimulation values of the burn polysomes were not different from those of the sham

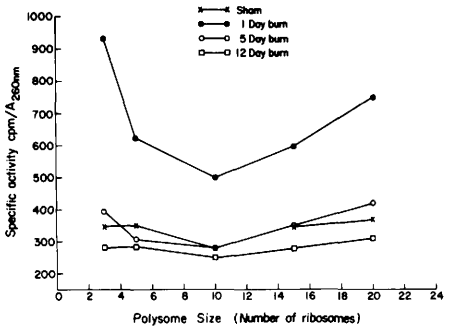

FIG. 2. Specific activity versus polysome size. The specific activities of selected polysome size classes were calculated and plotted against approximate polysome ribosome number for 1-, 5-, and 12-day postburn and sham-burn polysomes. The polysomes were isolated and specific activities were calculated as described in Figs. 1a and b. Sham (×——×), 1-day burn (●——●), 5-day burn (○——○), 12-day burn (□——□).

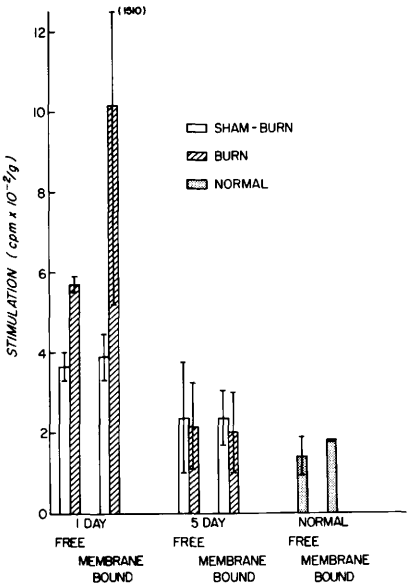

FIG. 3. Stimulation of protein synthesis. Free and membrane-bound polysomes were isolated from rat livers and tested in a cell-free protein synthesis system as described under Materials and Methods. The incorporation of [^{35}S]methionine into acid-insoluble products was measured and cpm/g liver for each polysomal fraction was determined. Mean stimulations over blank for three separate animals were calculated using free and membrane-bound polysomes isolated 1- and 5-day postburn. Error bars indicate ±SD. Sham ☐, burn ▨, and normal ⁙.

except in the largest of the free fractions where the burn was less than the sham value.

Discussion. The incorporation of [^{3}H]-leucine into newly synthesized proteins in rat liver increased dramatically 24 hr following burn injury. By 5 days, this increase in rates of protein synthesis returned to sham values. In an effort to determine what factors are involved in these changes in protein synthesis, we isolated free and membrane-bound polysomes from burn and sham animals and tested their protein synthesizing capacities in a rabbit reticulocyte cell-free protein synthesizing system. The results were similar to the studies *in vivo*, i.e., the ability of polysomes isolated from rat liver 24-hr postburn to synthesize proteins *in vitro* was increased approximately 150–260% over sham polysomes and returned to sham values by 5-day postburn. Following an extensive full-thickness skin burn, the body progresses rapidly into a hypermetabolic state. The increase in translation of proteins *in vivo* following burn injury is consistent with this clinical observation. Excessive breakdown of the peripheral muscle mass, enhanced gluconeogenesis, and increased circulating catecholamines are manifestations of the immediate postburn trauma period (14). Elevated protein synthesis in the liver may be in response to the increased albumin synthesis needed to replace that lost via the burn wound (15) and altered compartmentalization (16). A progressive decrease in albumin and increases in certain other serum proteins have been observed (17) along with increases in protein and RNA synthesis rates (16, 18, 19). Selective alterations in the expression of hepatic mRNA have also been suggested following protein deprivation (5). The alterations described here may be brought about in part by the selective increases *in vivo* in the specific activities of certain polysome size classes following burn injury. Since these increases differ in each size group, any alteration in the specific activity of the precursor pool would require selective changes in discrete compartments. A second possibility might be the recruitment of previously inactive ribosomes. However, increased specific activity of postburn polysomes were

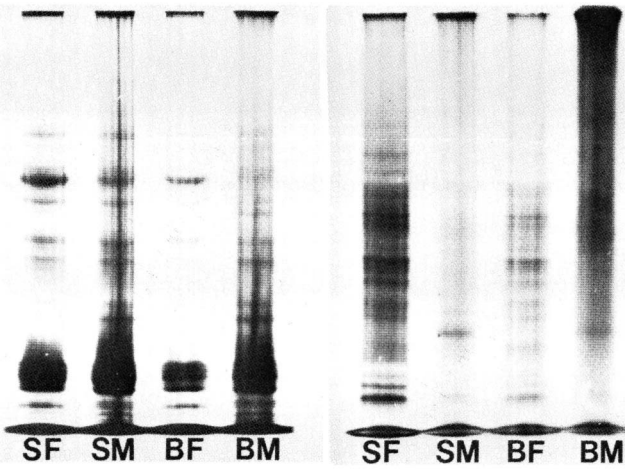

FIG. 4. (a) SDS–polyacrylamide gel electrophoresis of rabbit reticulocyte lysate products. Products from the rabbit reticulocyte lysate system described under Materials and Methods were electrophoresed on 10% polyacrylamide gels. Lanes 1–4, products from polysomes isolated 1-day post-burn. SF, sham free; SM, sham membrane bound; BF, burn free; BM, burn membrane bound. (b) Autoradiography of SDS–polyacrylamide gel of rabbit reticulocyte products. The gel shown in (a) was dried and electrophoresed as described under Materials and Methods.

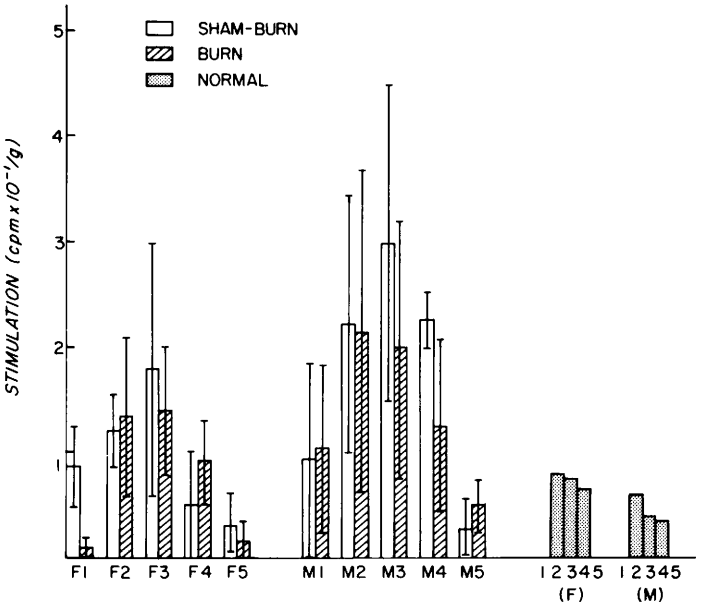

FIG. 5. Stimulation of protein synthesis by fractionated polysomes. Free and membrane-bound polysomes were isolated from rat livers, fractionated on linear sucrose gradients, and tested in the cell-free protein synthesis system as described under Materials and Methods. Incorporation of [^{35}S]methionine into acid-insoluble products was measured and cpm/g liver for each polysomal fraction was determined. Mean stimulations over blank were calculated for five subfractions of free (F) and membrane-bound (M) polysomes isolated from three 1-day burn and three sham rats and from normal rats (normal rat livers were pooled). Errors bars indicate ±SD. Approximate S values for each fraction are as follows: F1 and M1, 370–500 S; F2 and M2, 280–370 S; F3 and M3, 190–280 S; F4 and M4, 100–190 S; and F5 and M5, up to 100 S. Sham ☐, burn ▨, and normal, ⁘. Values below blank were considered zero.

apparent both *in vivo* and *in vitro* suggesting a mechanism involving altered rates of translation analogous to that seen in α- and β-globin polysomes (6–8). The differences between burn and sham subfractionated polysomes *in vitro* are not significant. Thus, it appears that a stimulatory factor which may be present in the total hepatic polysomal fractions from the burn animals, causing them to be more active than shams, may be eliminated by the subfractionation procedure.

Summary. Increases in the nascent protein specific activities of certain polysome size groups have been found in rat livers following a 20% full-thickness skin burn. The ratio of monosomes to polysomes was approximately 0.10 for the sham-burn animals and 0.06, 0.08, and 0.12 for the 1-, 5-, and 12-day postburn animals, respectively. The specific activities increased 70–170% above sham values 24-hr postburn and returned to sham levels by 5-day postburn. Studies *in vitro,* using a rabbit reticulocyte-message-dependent lysate system produced similar results. Free polysomes isolated from postburn rat livers stimulated protein synthesis in the cell-free system to a greater extent than the corresponding shams 24 hr following burn injury. By 5 days the stimulation from the burn polysomes was not statistically different from that of the shams. Separating the polysomes into narrower size classes eliminated the stimulatory effect observed *in vitro* at 1-day postburn. These data are consistent with the formation of a polysome-associated factor induced by burn trauma that potentiates protein synthesis, but which is unstable to polysome subfractionation.

We are indebted to Ms. Karen R. Lawley for carrying out amino acid analyses and Drs. Ajit Kumar and Daniel Malamud for helpful discussions.

1. Stein, T. P., Oram-Smith, J. C., Wallace, H. W., and Leskiw, M. J., J. Surg. Res. **21**, 201 (1976).
2. Stein, T. P., Leskiw, M. J., Wallace, H. W., and Oram-Smith, J. C., Amer. J. Physiol. **233**, E348 (1977).
3. Morgan, E. H., and Peters, T., J. Biol. Chem. **246**, 3500 (1971).
4. Garlick, P. J., Millward, D. J., James, W. P. T., and Waterlow, J. C., Biochim. Biophys. Acta **414**, 71 (1975).
5. Pain, V. M., Clemens, M. J., and Garlick, P. J., Biochem. J. **172**, 129 (1978).
6. Lodish, H. F., and Jacobsen, M., J. Biol. Chem. **247**, 3622 (1972).
7. Lodish, H. F., and Nathan, D. G., J. Biol. Chem. **247**, 7822 (1972).
8. Lodish, H. F., Nature (London) **251**, 385 (1974).
9. Ramsey, J. C., and Steele, W. J., Biochemistry **15**, 1704 (1976).
10. Pelham, H. R. B., and Jackson, R. J., Eur. J. Biochem. **67**, 247 (1976).
11. Hunt, T., and Jackson, R. J., *in* "Modern Trends in Human Leukaemia" (R. Neth, R. C. Gallo, S. Spiegelman, and F. Stohlman, eds.), pp. 300–307. J. F. Lehmanns Verlag, Munich (1974).
12. Darnbrough, C. H., Legon, S., Hunt, T., and Jackson, R. J., J. Mol. Biol. **76**, 379 (1973).
13. Laemmli, U. K., Nature (London) **227**, 680 (1970).
14. Wilmore, D. W., Long, J. M., Mason, A. D., Skreen, R. W., and Pruitt, B. A., Ann. Surg. **180**, 653 (1974).
15. Birke, G., Liljedahl, S.-O., Plantin, L.-O., and Reizenstein, P., Acta Chir. Scand. **134**, 27 (1967).
16. Brown, W. L., Bowler, E. G., Mason, A. D., and Pruitt, B. A., Amer. J. Physiol. **231**, 476 (1976).
17. Prendergast, J. J., Fenichel, R. L., and Daly, B. M., Arch. Surg. **64**, 733 (1952).
18. Kien, C. L., Young, V. R., Rohrbaugh, D. K., and Burke, J. F., Ann. Surg. **187**, 383 (1978).
19. Abakumova, O. I. U., and Kutsenko, N. G., Vopr. Med. Khim. **22**, 826 (1976).
20. Brown, P. C., and Papaconstantinou, J., Biochem. Biophys. Res. Commun. **76**, 121 (1977).

Received May 23, 1979. P.S.E.B.M. 1980, Vol. 164.