INCORPORATION OF PHENYLALANINE INTO PROTEIN BY MICROSOMES FROM REGENERATING RAT LIVER[1]

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As the problem of growth of regenerating rat liver has become more thoroughly explored by *in vitro* techniques, it has become increasingly evident that a number of the components necessary for protein synthesis are dramatically altered. Since it has been reported that nuclear RNA[3] polymerase activity is increased following partial hepatectomy, (Tsukoda and Lieberman, 1965a) a search for increased mRNA should be productive. Indeed, recent reports have demonstrated an increased heavy polyribosome content, (Tsukoda and Lieberman, 1965b; Cammarano, Giudice, and Lukes, 1965) and a factor with messenger like activity has been extracted from microsomes (Hoagland, 1961). At variance with these findings is the report that Poly U stimulated microsomes from livers of hepatectomized animals more than microsomes from control animals (Cammarano, Melli, and Novelli, 1965). If regenerating microsomes contained more mRNA the opposite effect would be expected, and was found by other investigators (Campbell and Cooper, 1963). Since a difference in amino acid activation might account

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[3]Abbreviations used in the text are GTP, guanosine 5'-triphosphate; Poly U polyuridylic acid; Poly A, polyadenylic acid; Poly C, polycytidylic acid; RNA, ribonucleic acid; mRNA, messenger RNA; sRNA, soluble cytoplasmic RNA.

for the apparent discrepancy,(Hultin and von der Decken, 1958) the present study was undertaken to investigate the effect of Poly U on C^{14}-phenylalanine transfer from aminoacyl-sRNA to protein in the presence of microsomes from regenerating livers (H mic.) and livers from sham operated animals (S mic.). These experiments confirm that H mic. are twice as active as S mic. when compared on the basis of gram equivalents of fresh tissue, and that differences in activity are abolished in the presence of the synthetic polynucleotide.

METHODS

Following 70% hepatectomy (Higgins and Anderson, 1931) and sham laparotomy, the animals were left fasting and sacrificed 24 hours later. Microsomes were prepared by the method of Hoagland et al. (1964a) as the pellet from a 15 minute 59,000 x g centrifugation after mitochondria and nuclear debris had been removed. A '100,000 g supernatant' from one hour of centrifugation was used as the source of transfer enzymes following two hours of dialysis and 24 hours of freezing. Aminoacyl-sRNA was charged with C^{14}-phenylalanine and 19 other C^{12} amino acids and was isolated, incubations were performed, and protein fraction analysed according to the methods of Liao and Williams-Ashman (1962).

RESULTS AND DISCUSSION

In Table 1 is shown that the complete system is stimulated seven

Table I

System	Radioactivity transferred to protein fraction (cpm)	
	No Poly U	Plus Poly U
Complete	820	6196
Plus Poly A (100 μg)	755	1016
Plus Poly C (100 μg)	678	5333
Plus C^{12}-phenylalanine (1 μ mole)	782	5534

The assay system was as described in methods. Incubations were for 40 minutes. Each tube contained aminoacyl-sRNA (142 μg, 8360 cpm), liver microsomes (0.1 gm. eq.) and '100,000 g supernatant' (0.05 gm. eq.) prepared from normal rats. Poly U (100 μg) was added to indicated tubes.

fold by Poly U but not by Poly A which inhibits Poly U stimulation. Poly C has no effect on the system. The addition of C^{12}-phenylalanine does not inhibit amino acid transfer. This indicates that the radioactivity in the protein fraction is comming from the previously prepared aminoacyl-sRNA and that the stimulation of incorporation by Poly U is because it serves as a synthetic messenger. The '100,000 g supernatant' has virtually no capacity to activate amino acids. Figure 1 demonstrates that the extent of incorporation is limited by the amount of microsomes present and that for a given amount, the H mic. are more active than S mic. This difference is abolished at every level by the addition of Poly U.

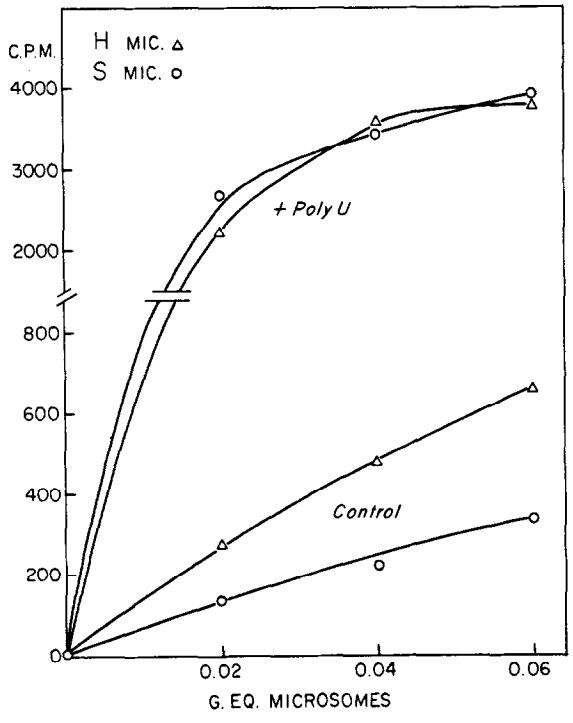

Figure 1. The effect of Poly U on the transfer of C^{14}-phenylalanine from aminoacyl-sRNA to protein fraction by microsomes from livers of hepatectomized (triangles) and sham operated rats (circles) in the presence of '100,000 g supernatant' (0.05 gm. eq.) from livers of normal animals. Assay for incorporating ability was as described in the text. Aminoacyl-sRNA (135 μg, 7680 cpm) was added to each tube and Poly U (100 μg) was added to indicated tubes.

When aminoacyl-sRNA and Poly U are added after 20 minutes of incubation (fig. 2) , the degree of stimulation of incorporation is exactly the same as when an equal amount of these constituents is added to the original incubation mixture at time zero. Addition of the aminoacyl-sRNA alone at 20 minutes does not stimulate incorporation demonstrating that deacylation of the aminoacyl-sRNA does not limit the extent of incorporation. Since GTP is present in excess, the presence of an inhibitor on normal microsomes described by Hoagland et al. (1964b) would be masked.

The results of the current investigations clearly indicate that Poly U stimulates H mic. and S mic. to similar levels of incorporation.

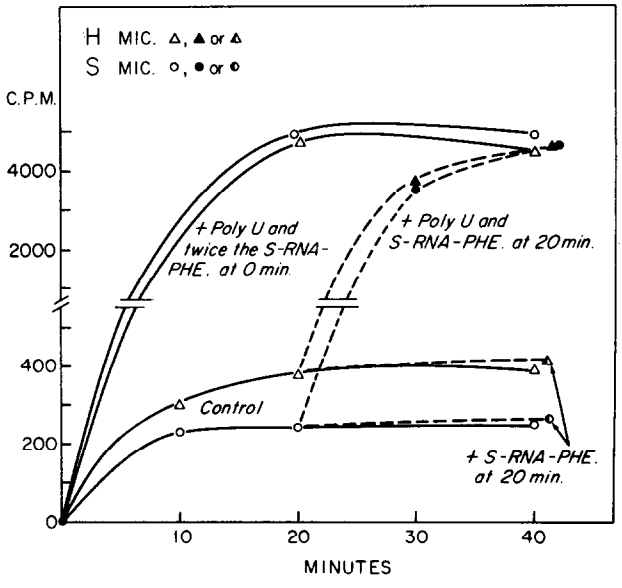

Figure 2. The stimulation of amino acid incorporation by the addition of Poly U (100 μg) and aminoacyl-sRNA at various times during the incubation of microsomes (0.05 gm. eq.) from livers of hepatectomized (triangles) and sham operated rats (circles) in the presence of '100,000 g supernatant' (0.05 gm. eq.) from livers of normal rats. Where indicated Poly U and aminoacyl-sRNA (127 μg, 7100 cpm) were added to the reaction mixtures at zero time (+ POLY U AND TWICE THE S-RNA-PHE. AT 0 MIN.). Since an equal amount of aminoacyl-sRNA was already present in these tubes, the total content of this substrate was 252 μg and 14,200 cpm. Aminoacly-sRNA alone or with Poly U was also added where indicated to tubes that had already been incubating with a full complement of reactants for twenty minutes (respectively + S-RNA-PHE. AT 20 MIN.▲, ●, and + POLY U AND S-RNA-PHE. AT 20 MIN.▲, ●). After the additions thse vellels likewise contained 252 μg of aminoacyl-sRNA (14,200 cpm).

One explaination for these findings is that the extent of incorporation by the microsomes may be limited by the mRNA content , and that the vast excess of synthetic messenger provided by the addition of Poly U masks this difference. Analogous findings have been reported by workers studying the testosterone stimulated rat ventral prostate in which increased ribosomal mRNA content has been implied from results similar to these (Silverman et al., 1963) and most likely reflects the increased RNA polymerase activity described in that gland. (Hancock et al., 1962)

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REFERENCES

Cammarano, P., Giudice, G., and Lukes, B. (1965), Biochem. Biophys. Res. Comm., 19, 487.
Cammarano, P., Melli, M., and Novelli, G. D. (1965), Biochim. Biophys. Acta, 108, 329.
Campbell, P. N., and Cooper, C. (1963), Biochem. J., 89, 94p.
Hancock, R. L., Zelis, R. F., Shaw, M., and Williams-Ashman, H. G., Biochim. Biophys. Acta (1962), 55, 257.
Higgins, G. M., and Anderson, R. M., A. M. A. Arch. Pathol. (1931), 12, 186.
Hoagland, M. B. (1961), Cellular Regulatory Mechanisms, Cold Spring Harbor Symposia, 26, 153.
Hoagland, M. B., Scornick, O. A., and Cassidy, L. (1964a),Federation Proc., 23, 316.
Hoagland, M. B., Scornick, O. A., and Pfefferkorn, L. C. (1964b), Proc. Natl. Acad. Sci. U. S., 51, 1184.
Hultin, T., and von der Decken, A.(1958), Exptl. Cell Research, 15, 581.
Liao, S., and Williams-Ashman, H. G. (1962), Proc. Natl. Acad. Sci. U. S., 48, 1956.
Silverman, D. A., Liao, S., and Williams-Ashman, H. G.,(1963), Nature, 199, 808.
Tsukoda, K., and Lieberman, I. (1965a), J. Biol. Chem., 240, 1731.
Tsukoda, K., and Lieberman, I. (1965b), Biochem. Biophys. Res. Comm., 19, 702.