INCREASED PROTEIN SYNTHETIC CAPACITY IN VITRO OF RAT LIVER ROUGH ENDOPLASMIC RETICULUM FOLLOWING STARVATION

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SUMMARY: There is a dramatic rise in the protein synthetic activity of rough endoplasmic reticulum (RER) of liver from starved and starved-refed rats, compared to normal controls. This observation is similar to the doubling of protein synthetic activity of rat liver RER observed 20 hours after partial hepatectomy (1). Oxidised glutathione (GSSG), a potent inhibitor of protein synthesis by normal RER, was a much less effective inhibitor when assayed with RER from the livers of starved rats, this again is in parallel with our earlier observations in regenerating liver. This common pattern of response is discussed in terms of a cellular control system which responds to changes in the cytoplasmic sulphydryl concentration.

INTRODUCTION: We have recently shown (1) that regeneration causes liver microsomes to become twice as active *in vitro* in protein synthesis as the normal controls.

Starvation, another stress load, has been used in the studies presented here to generalize these earlier conclusions concerning the role of cytoplasmic control in protein synthesis.

Our results indicate that five days of starvation increases the activity of the rough endoplasmic reticulum by approximately 75 %; 4 days' starvation followed by refeeding causes a 106 % increase. In agreement with earlier work, where the more active, regenerating microsomes were more resistant to oxidized glutathione (GSSG) inhibition than the control, the microsomes from starved-refed rats were more resistant than those from the starved ones. These in turn were more resistant than the control.

MATERIALS AND METHODS: Starvation and Refeeding. Female Sprague-Dawley rats (Charles River Laboratories) of 125 g were starved (but had access to water ad libitum) for 5 days, or alternatively were starved for 4 days and allowed access to food from 5 p.m. until decapitation at 8 a.m. the next day. All animals were kept on a regular 12-hour light and 12-hour dark schedule.

The other techniques involved in these experiments are given in detail in reference 1, but will be summarised here.

Processing of livers: Livers were removed and rapidly cooled on ice. After homogenisation in two volumes of 0.25 M Succrose, 50 mM Tris-HCl, pH 8 @ 4° C, 25 mM KCl, 5 mM $MgCl_2$ (TKM) the suspension was centrifuged for 10 minutes at 15,000 g, and the supernatant treated as described below.

For pH 5 Supernatant: The supernatant was centrifuged for one hour at 100,000 g. The supernatant from this centrifugation was brought to pH 5.2 with acetic acid and centrifuged for 15 minutes at 15,000 g. This supernatant was restored to pH 7.5 with 1MKOH and passed through a Sephadex G-25 column of appropriate dimensions, which had been pre-equilibrated with 50 mM Tris-HCl pH 8 @ 4° C. The column effluent will be referred to as pH 5 supernatant or pH 5 SN.

For microsomes: The supernatant was centrifuged 20 minutes at 170,000 g. The pellet was resuspended in 1.53 M Sucrose TKM and centrifuged 1 hour at 170,000 g. The upper and lower regions were discarded and the central cloudy suspension was diluted 1:1 with sucrose-free TKM and centrifuged for 1 hour at 170,000 g. The pellet obtained is referred to as microsomes in this paper.

Cell free system for transfer assay: In a 25 µl assay the concentrations were Tris-HCl, 50 mM; $MgCl_2$, 6 mM; NH_4Cl, 80 mM; 15 µg ribosomal RNA; 0-60 µg pH 5 SN protein; phosphoenol pyruvate, 10 mM; GTP, 0.01 mM; pyruvate kinase, 1.25 µg; tRNA mixture containing [^{14}C]leu tRNA, 0.25 µl (1040 d.p.m./µl, 21 d.p.m. per p.mole tRNA). Incubation was for 10 minutes

at 30° C. Reaction was terminated by adding 20 µl 1 MKOH, followed by 10 minutes incubation at 37° C to hydrolyse unreacted [^{14}C]leu tRNA. 3 ml 5 % TCA was then added to precipitate protein, which was collected and washed on glass-fiber filters, and then assayed for radioactivity in a scintillation counter.

RESULTS: In a previous paper (1) we have shown that (a) the conditions used for these experiments are such that we are still on the linear part of the reaction versus time curve. Thus the results are measures of initial rates of amino acid transfer from [^{14}C]amino-acyl-tRNA into protein. (b) the GTP requirement of the system is 0.01 mM and (c) the system is highly dependent on added pH 5 SN. Microsome activity is reported in terms of p.moles of total amino acid incorporated in ten minutes at 30° C per mg of ribosomal RNA.

Activity of pH 5 SN from normal, starved, and starved-refed animals: In agreement with Migliorini and Manchester (2) we found that on a per weight protein basis the pH 5 SN from starved animals was less active than material from the controls. In addition, we found that the pH 5 SN from starved-refed rats were more active than the normal and approached that of regenerating pH 5 SN. These differences were particularly evident at subsaturating levels of pH 5 SN.

The data presented in Table I are the mean of two independent experiments each performed in triplicate. The data have been derived from a large number of pH 5 SN concentration versus activity plots, a typical example of which is shown in Fig. I. The points used for comparison were those corresponding to a 20-µg pH 5 SN protein input per assay.

Activity of microsomes from normal, starved and starved-refed rats: The marked differences in activity between the different classes of microsome are clearly demonstrated in Table I. In the presence of all three types of pH 5 SN the order of activity is $M_{s-r} > M_s > M_n$.

Resistance of different microsome types to GSSG inhibition: In our earlier

Table I

In vitro protein synthesis activity of microsomes and pH 5 SN from normal, starved, and starved-refed rats

pH 5 SN Source	Microsomal type		
	normal	starved	starved-refed
	p.moles amino acid incorporated per mg rRNA		
Normal	104	199	231
Starved	62	151	197
Starved-refed	154	210	233
Sum	320	560	661
Relative activity	1	1.75	2.05

The standard assay system as described in Methods was used with 20 µg of pH 5 SN protein from normal, starved or starved-refed animals used with each type of microsome.

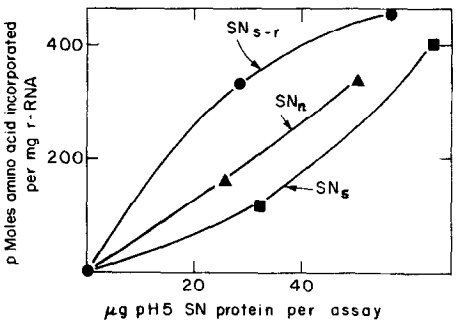

Fig. 1: Normal microsomes were incubated as described in the methods section with pH 5 SN fractions from normal (n), starved (s) and starved-refed (s-r) animals.

paper (1) we described a marked inhibitory effect of 0.1 mM GSSG on the amino acid incorporation activity of microsomes. Microsomes from regenerating livers were twice as active in *in vitro* protein synthesis as control microsomes, and were only 50 % inhibited by GSSG, while the controls were

Table II

Inhibition of amino acid incorporation in vitro by 0.1 mM GSSG

pH 5 SN Source	Microsome type		
	normal	starved	starved-refed
	p.moles amino acid incorporated per mg rRNA		
Normal	18.7	44.0	82.5
Starved	15.4	30.8	89.5
Starved-refed	15.8	77.0	135.0
Sum	49.9	151.8	307.0
% Inhibition by GSSG*	84 %	63 %	53 %
Relative activity	1	3.03	6.15

The activities of microsomes and pH 5 SN from normal, starved, and starved-refed animals are presented.
* The degree of inhibition was obtained by using the sum figures in Table I as representing 100 % activity for each class of microsome.

80 % inhibited. Thus there appeared to be a correlation between the protein synthetic capacity and resistance to GSSG inhibition. In order to test this correlation, we investigated the degree of resistance to GSSG of the microsomes used in these experiments. The results of these experiments appear in Table II. In agreement with our earlier findings (1), M_n are inhibited 84 % by 0.1 mM GSSG. Starved microsomes are inhibited 63 %, and starved-refed are inhibited to the extent of ~ 53 %. These differences are significant since similar results have been obtained in eight out of nine experiments.

DISCUSSION: Sox and Hoagland (3) reported that liver microsomes from starved-refed rats were more active in protein synthesis than those from

starved animals. This activity change has been confirmed in these studies and also has been put in perspective by relating the data to normal controls. The decrease in capacity of cell sap to support protein synthesis following starvation has been studied by Migliorini and Manchaster (2). We have confirmed their observation and extended it to demonstrate a large increase in the activity of the pH 5 SN following 15 hours of refeeding.

Our preliminary experiments using sham operation demonstrated that the trauma of laparotomy induced a short-term (15 hour) period of high microsome activity which was sustained for longer periods only if partial heytectomy was also performed. We thus concluded that the initial hight activity of the microsomes was a stress response. Tsukada et al. (4) have observed a similar increase in activity following acute stress caused by intraperitoneal injection of Celite. The finding that starvation, another form of stress, increases the activity of microsomes therefore lends support to our earlier hypothesis. The further increase in activity observed upon refeeding after 4 days starvation suggests that the massive metabolic load imposed on the liver by ingestion of food is an even greater stress on the organ.

Reid et al. (5) have described studies on the effects of starvation on livers of suckling and weanling rats. They report that deoxycholate- (DOC) treated polysomes derived from these tissues have diminished activity per unit RNA after starvation, but do not provide data from non-detergent-treated microsomes. We suspect that these results may be attributable to changes brought about by the detergent on the two types of rough endoplasmic reticulum. We have previously noted significant differences in the properties of microsomes and the polysomes derived from them by DOC, so this apparent contradiction in results mediated by DOC is not unique to this system.

The same authors present data using total postmitochondrial supernatant which show that material derived from starved rats is less active than

from controls in amino acid incorporation, expressed per unit RNA. Since Reid et al. did not correct also for protein, these observations may be attributed to the lower activity of the cell sap (2) or more specifically the pH 5 SN fraction which we have described here.

We conclude, therefore, that the data obtained here support our earlier results (1) and broadens the validity of the redox control hypothesis.

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REFERENCES:

1. R. D. Nolan and M. B. Hoagland, Biochim. Biophys. Acta 247, 609 (1971)
2. R. H. Migliorini and K. L. Manchester, FEBS Letters 13, 140 (1971)
3. H. C. Sox and M. B. Hoagland, J. Mol. Biol. 20, 133 (1966)
4. K. Tsukada, T. Moriyama, O. Doi and I. Lieberman, J. Biol. Chem. 243, 1152 (1968)
5. I. M. Reid, E. Verney and H. Sidransky, J. Nutr. 100, 1149 (1970)