Shyamala, G., and Gorski, J. (1969), *J. Biol. Chem. 244*, 1097.
Sluyser, M. (1966), *J. Mol. Biol. 19*, 591.
Spelsberg, T. C., Steggles, A. W., and O'Malley, B. W. (1971), *J. Biol. Chem. 246*, 4188.
Watanabe, H., Nicholson, W., and Orth, D. N. (1973a) *Endocrinology 93*, 411.
Watanabe, H., Orth, D. N., and Toft, D. O. (1973b), *J. Biol. Chem. 248*, 7625.

Inhibition of *in Vitro* Amino Acid Incorporation by Sodium Selenite†

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ABSTRACT: Nanomole quantities of sodium selenite completely inhibited amino acid incorporation in a system consisting of free polyribosomes and a 150,000*g* supernatant prepared from rat liver, fortified with cofactors. The 150,000*g* supernatant contained a factor required for maximum expression of the inhibitory effect. This factor could be replaced by thiols, *e.g.*, glutathione. Incorporation of leucine from L-[U-^{14}C]-leucyl-tRNA into protein by free polyribosomes and partly purified transferases was not at all affected by Na_2SeO_3, but 100% inhibition was obtained by Na_2SeO_3 in the additional presence of glutathione. A reaction product between Na_2SeO_3 and glutathione, which could be the selenotrisulfide derivative of glutathione (selenodiglutathione, GSSeSG), was found to be a very potent inhibitor of amino acid incorporation, abolishing the amino acid incorporation process beyond aminoacyl-tRNA formation.

Various hepatocarcinogens cause peroxidation of the unsaturated fatty acids of liver endoplasmic reticulum membranes (Rao and Recknagel, 1968; Jose and Slater, 1970). Lipoperoxidation could be involved in the acute toxic effects of the carcinogens on liver, and accompany liver carcinogenesis as a symptom of the metabolic production of free radicals from these carcinogens (Recknagel, 1967).

Seleno compounds have been shown to act as antioxidants and free-radical scavengers (Tappel, 1965). During experiments in which antioxidants were applied to counteract liver microsomal lipoperoxidation, we found that sodium selenite exerted a pronounced inhibition of amino acid incorporation by membrane-bound polyribosomes from rat liver *in vitro*. In order to exclude membrane effects, the inhibitory activity of selenite was studied on free polyribosomes, and the results are reported in the present paper. We were motivated to investigate this aspect of the biological action of selenite in view of the known toxicity of this and other seleno compounds (Rosenfeld and Beath, 1964; Harr and Muth, 1972), and the tumor growth inhibitory properties of some seleno compounds (Harr and Muth, 1972; Shapiro, 1972).

Materials and Methods

Chemicals. DL-[1-^{14}C]Leucine was obtained from the Radiochemical Centre, and L-[U-^{14}C]leucyl-tRNA from New England Nuclear Corp. Glutathione was purchased from Sigma, oxidized glutathione, phosphoenolpyruvate, and pyruvate kinase from Boehringer, sodium selenite from Merck, and Dowex 50W-X4 from Bio-Rad.

Preparation of Free Polyribosomes. Free polyribosomes were isolated according to the method of Bloemendal *et al.* (1967).

Preparation of the 150,000g Supernatant. Rat liver was homogenized in 2 ml of ice-cold buffer A per g (wet weight) of tissue. Buffer A consisted of 50 mM Tris-HCl (pH 7.6), 25 mM KCl, 10 mM $MgCl_2$, and 0.35 M sucrose. The homogenate was centrifuged at 15,000*g* for 10 min and the 15,000*g* supernatant was centrifuged for 1 hr at 150,000*g*. As indicated and described in the text, the 150,000*g* supernatant was submitted to gel filtration over Sephadex G-25.

Preparation of Aminoacyl Transferases. The method of Gasior and Moldave (1965) has been followed partially. The pH of the 150,000*g* supernatant was lowered to 5.2 by adding 1 N acetic acid. The resulting suspension was kept in an ice bath for 15 min and then centrifuged at 10,000*g* for 10 min. The remaining supernatant was passed through a column of Sephadex G-25 (1.7 × 30 cm), equilibrated and eluted with buffer A. The first peak, containing the transferases, was used in the amino acid incorporation experiments.

Preparation of Aminoacyl-tRNA. The method of Bloemendal *et al.* (1962) has been followed partially. Equal volumes of 105,000*g* rat liver supernatant and a phenol–cresol mixture as described by Kirby (1965) were mixed and shaken for 10 min at 4°. Following centrifugation for 20 min at 35,000*g* the two layers were separated and the phenol layer was extracted once with an equal volume of a 10^{-4} M EDTA (pH 4.5) solution. The water layers were combined and a twofold volume of 96% ethanol supplemented with 2% ammonium acetate was added. The suspension was kept at −20° for at least 2 hr and centrifuged. The RNA precipitate was dissolved in water and centrifuged and the RNA solution was precipitated once with ethanol. After dissolving the precipitate in water, the solution was applied to a column of Sephadex G-25. The first peak eluted contained aminoacyl-tRNA. The final solution was concentrated by lyophilization.

Incubation of [^{14}C]Leucine with Polyribosomes. The incubation mixture contained 0.5 μmol of ATP, 0.25 μmol of GTP, 5.0 μmol of phosphoenolpyruvate, 25 μg of pyruvate kinase, 0.175 μCi of DL-[1-^{14}C]leucine (sp act. 7 Ci/mol), 5

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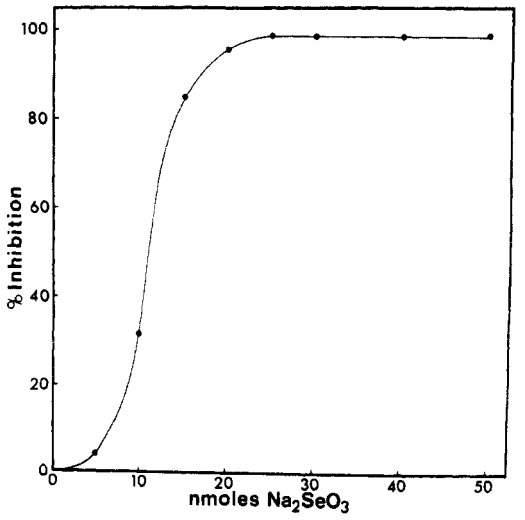

FIGURE 1: Inhibition of [^{14}C]leucine incorporation as a function of the sodium selenite concentration. Incubation was performed with free polyribosomes and 150,000*g* supernatant; final volume 1 ml. For further details see Materials and Methods.

absorption units at 260 nm of free polyribosomes, 0.10 ml of 150,000*g* rat liver supernatant, or 0.15 ml from the first peak after gel filtration of this supernatant by Sephadex G-25 as indicated in the text (Figure 2). In the latter case 0.05 μmol of the other 19 nonlabeled amino acids was added.

Incubation of [^{14}C]*Leucine with tRNA.* The incubation mixture contained 1 μmol of ATP, 200 μg of stripped tRNA, 0.175 μCi of DL-[1-^{14}C]leucine (sp act. 7 Ci/mol), and 0.15 ml from the first peak after gel filtration of 150,000*g* supernatant by Sephadex G-25. The tRNA was stripped of attached amino acids by incubation in 0.2 M sodium carbonate buffer of pH 9.5 for 30 min. After incubation the mixture was cooled in ice and 1 ml of 10% Cl_3CCOOH supplemented with 1 μmol of cold DL-leucine and 10 mg of Hyflo was added. The precipitate was collected by centrifugation, resuspended in 5% Cl_3CCOOH, collected on a paper filter (pore size 15–40 mμ), and washed with 5% Cl_3CCOOH and 96% alcohol.

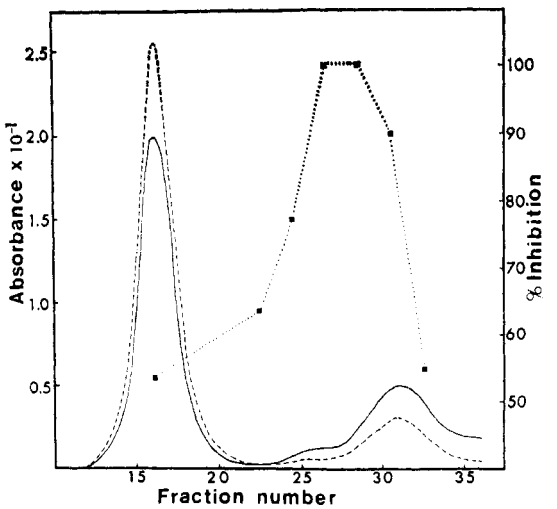

FIGURE 2: Effect of various fractions obtained from the rat liver 150,000*g* supernatant on the inhibition of amino acid incorporation by sodium selenite. Fractionation of 4 ml of 150,000*g* supernatant was performed by gel filtration on a column of Sephadex G-25 medium (1.7 × 30 cm). Fractions of 30 drops were collected and measured at 260 nm (——) and 280 nm (----). Fractions 15, 16, and 17 were pooled and 0.15 ml was used as the enzymic source in the incorporation experiments. In addition 0.15 ml from two adjacent pooled fractions, starting with fractions 22 and 23, was added. The percentage inhibition obtained by 50 nmol of sodium selenite in each experiment is expressed relative to the incorporation obtained in the corresponding incubations without sodium selenite and is plotted as a function of the fraction number (■).

TABLE I: Effect of Thiols on Sodium Selenite Inhibition of Amino Acid Incorporation by Free Polyribosomes Incubated with Fractionated 150,000*g* Supernatant.[a]

Addition	cpm		%
	Control	Na_2SeO_3[c]	Inhibition
None	880	331	62
Glutathione[b]	929	10	99
Cysteine[b]	864	19	98
2-Mercaptoethanol[b]	886	23	97

[a] Final volume 1 ml. Incubation conditions are described in Materials and Methods, fractionation of 150,000*g* supernatant in Figure 2. [b] 0.1 μmol. [c] 50 nmol.

Incubation of [^{14}C]*Leucyl-tRNA with Polyribosomes.* The incubation mixture contained 0.25 μmol of GTP, 5 μmol of phosphoenolpyruvate, 25 μg of pyruvate kinase, 13 μg of L-[U-^{14}C]leucyl-tRNA (sp act. 0.355 μCi/mg), 97 μg of aminoacyl-tRNA, 5 absorption units at 260 nm of free polyribosomes, and aminoacyl transferases corresponding to 1.5 mg of protein. For the determination of protein the method of Lowry *et al.* (1951) was used with bovine serum albumin as a standard.

Incubation in General. The ionic concentrations were adjusted to 50 μmol of Tris-HCl (pH 7.6), 70 μmol of KCl, 6 μmol of $MgCl_2$, and 2 μmol of $CaCl_2$; final volume 1 ml. Incubations were carried out for 30 min at 37°. Radioactivity was assayed as described previously (Vernie *et al.*, 1971).

Results

Inhibition of Amino Acid Incorporation by Sodium Selenite in Vitro, and the Involvement of Thiols in This Process. Using free polyribosomes, the 150,000*g* supernatant derived from rat liver as a source of enzymes for amino acid incorporation and the required cofactors, the effect of increasing amounts of selenite on DL-[1-^{14}C]leucine incorporation was studied. Under the conditions specified in Figure 1, 50% inhibition of amino acid incorporation was obtained by as few as 11 nmol of sodium selenite/ml of incubation mixture (1.1×10^{-5} M), 100% inhibition being obtained by 25 nmol of selenite. In contrast, the same amount of sodium sulfite did not affect the incorporation. Hardly any effect was found with 25 nmol of cycloheximide. The inhibitory effect of cycloheximide reached a plateau value of 85% at relatively high (1.5 μmol) concentrations.

When, instead of the 150,000*g* supernatant, the high molecular weight fraction obtained from the latter supernatant by gel filtration over Sephadex G-25 (fractions 15–17, in Figure 2) was used as a source of enzymes, a substantially lower inhibition of amino acid incorporation by 25 nmol of selenite was consistently observed, *i.e.* 55% on the average (range 49–66%). Increasing the amount of selenite did not further affect the measure of inhibition. This result suggested that an additional factor required for optimal inhibition by selenite was present in the 150,000*g* supernatant, and had been

TABLE II: Effect of Sodium Selenite and/or Glutathione on Aminoacyl-tRNA Formation.[a]

Addition	Expt 1[b]	% Inhibition[d]	Expt 2[c]	% Inhibition[d]
None	3393		3008	
0.1 μmol of glutathione	3365	0	2783	7
50 nmol of sodium selenite	2446	28	2638	12
0.1 μmol of glutathione + 50 nmol of sodium selenite	2396	29	2118	30

[a] Final volume 1 ml. Incubation was performed with 0.15 ml of Sephadex G-25 fractions 15–17 (Figure 2). Incubation conditions as described in Materials and Methods. [b] Nonlabeled amino acids were added. Radioactivity in counts per minute (cpm). [c] Without addition of nonlabeled amino acids. Radioactivity in cpm. [d] Inhibition expressed as percentage of the control without glutathione or sodium selenite.

removed by gel filtration. As shown in Figure 2 (■) the 55% inhibition produced by selenite in the latter system was raised to 100% by inclusion of either of the low molecular weight fractions 26 + 27 or 28 + 29 (0.15 ml) obtained by gel filtration from the original 150,000*g* supernatant. In the absence of selenite these fractions had no significant effect on amino acid incorporation. Thus, the 150,000*g* supernatant contains a low molecular weight factor that has to be present for selenite to exert its maximum inhibitory effect on amino acid incorporation.

Selenite is known to react with thiols (Ganther, 1968), and the low molecular weight factor in our system could be an SH-containing compound such as glutathione which is the main nonprotein sulfhydryl in liver (Jocelyn, 1972a). Accordingly, the effect of glutathione, cysteine, and 2-mercaptoethanol on the inhibition by selenite of the amino acid incorporating system consisting of free polyribosomes and the Sephadex G-25 fractions 15–17 (as a source of enzymes) was tested. As shown in Table I, the inhibition caused by selenite (50 nmol) was raised to 100% by the addition of 0.1 μmol of either one of these three thiols.

Since cysteine (50 nmol) was a component of the nonlabeled amino acid mixture present in the experiments in which the Sephadex G-25 fractions 15–17 were used as an enzyme source, the latter result predicts that omission of cysteine from this mixture should further reduce the inhibition caused by selenite. This proved to be the case since the inhibition of amino acid incorporation dropped from an average of 55 to 23–31%, whereas the omission of cysteine from the controls did not materially affect the level of incorporation as compared with that obtained in the presence of cysteine.

Effect of Sodium Selenite and/or Glutathione on the Formation of Aminoacyl-tRNA. In order to locate the site of inhibition of selenite (and the effect of added thiol) in the sequence of reactions involved in the incorporation of amino acid into protein, the formation of leucyl-tRNA from DL-[1-^{14}C]leucine was studied using the Sephadex G-25 fractions 15–17 as enzyme source in the absence or presence of the other nonlabeled amino acids. The latter were included to mimic the situation prevailing in the system used in the experiments of the previous section (cysteine effect). In the presence of the unlabeled amino acids, selenite (50 nmol) inhibited leucyl-tRNA formation from [^{14}C]leucine for 28–36%. The inclusion of glutathione (0.1 μmol) had no further effect (Table II). In the absence of amino acids, selenite caused a smaller inhibition of leucyl-tRNA formation, *i.e.* 12–20%, whereas inclusion of glutathione (or cysteine) now increased the inhibition to the level observed in the former experiment. It is concluded that aminoacyl-tRNA formation is only partially blocked by selenite and that this effect is dependent on the presence of thiol. Inhibition obtained in the absence of an added sulfhydryl compound could be due to such a compound persisting in the preparation.

TABLE III: Effect of Sodium Selenite and/or Glutathione on Leucine Incorporation from L-[U-^{14}C]Leucyl-tRNA into Protein by Free Polyribosomes.[a]

Addition	Expt 1[b]	Expt 2[b]
None	2084	2176
0.1 μmol of glutathione	2053	2271
25 nmol of sodium selenite	2132	2073
0.1 μmol of glutathione + 25 nmol of sodium selenite	55[c]	134[d]

[a] Final volume 1 ml. Incubation conditions as described in Materials and Methods. [b] Radioactivity in cpm. [c] Representing 97% inhibition as compared with control without glutathione or sodium selenite. [d] Representing 94% inhibition.

Effect of Sodium Selenite and/or Glutathione on the Incorporation of Amino Acid from Aminoacyl-tRNA into Protein. The effect of selenite and glutathione, either separately or combined, on the incorporation of leucine from L-[U-^{14}C]-leucyl-tRNA into protein by free polyribosomes fortified with partially purified transferases and cofactors (no SH compound added), is listed in Table III. When added singly, neither selenite nor glutathione was inhibitory, but in combination these compounds abolished the incorporation process.

The leucyl-tRNA incorporating system was used further to test the effect of the products obtained from the reaction between sodium selenite and glutathione.

Isolation and Effect of the Inhibitory Substance Resulting from Reaction between Na_2SeO_3 and Glutathione. According to Ganther (1971) and Sandholm and Sipponen (1973), the reaction products between glutathione and SeO_3^{2-} at a molar ratio not exceeding 4:1 and at acid or neutral pH are selenotrisulfide (= selenodiglutathione, GSSeSG) and oxidized glutathione (= glutathione disulfide, GSSG).[1]

Products were isolated according to the procedure described by Ganther (1971), slightly modified. Na_2SeO_3 and GSH were allowed to react in twice distilled water of pH ~5.2, reaction and subsequent steps being performed at room temperature. The reaction mixture was processed by chromatography on Dowex 50W-X4 as indicated in Figure 3. The absorbance at 240 nm showed the presence of two peaks, designated as

[1] Abbreviations used are: GSSeSG, selenodiglutathione; GSSG, glutathione disulfide; GSH, glutathione.

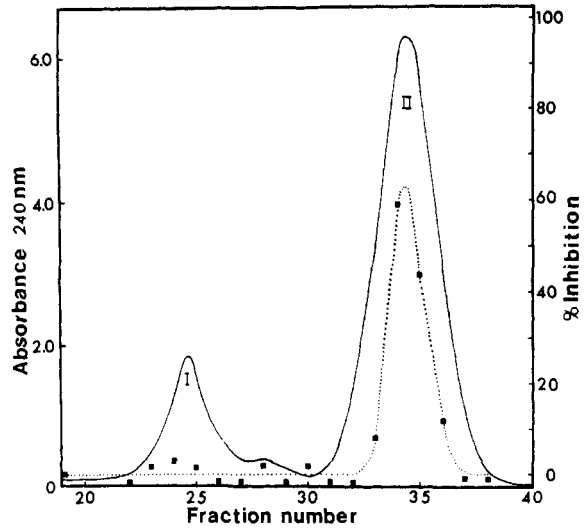

FIGURE 3: Isolation of the products resulting from the reaction between glutathione and sodium selenite, and their effect on amino acid incorporation. Glutathione (400 μmol) and 100 μmol of sodium selenite were separately dissolved in 3 ml of twice distilled water. The solution of glutathione was next added to that of sodium selenite under continuous mixing. After standing for 15 min, 4 ml of the reaction mixture was applied to a Dowex 50W-X4 column (2.5 × 60 cm). Equilibration of the column and elution of the reaction mixture were carried out with 0.1 M sodium acetate, adjusted to pH 4.7 with acetic acid, containing 0.01 M $NiCl_2$, according to Ganther (1971). Fractions of 8.5 ml were collected, the absorbance at 240 mμ was measured, and 0.01 ml of each fraction was added to the [^{14}C]-leucine incorporating system. Incubation was performed in a final volume of 1 ml containing free polyribosomes, partially purified transferases, and L-[U-^{14}C]leucyl-tRNA. The inhibition obtained is expressed as the percentage of the control performed with 0.01 ml of elution buffer, and is indicated as a function of the fraction number (■).

fraction I (fractions 24 and 25, Figure 3) and fraction II (fractions 34 and 35). The ultraviolet absorption spectra of fractions I and II were identical with those of GSSG and that assigned by Ganther (1971) to GSSeSG, respectively.

The inhibitory effect of the eluate as a function of the fraction number was tested by the addition of 0.01-ml portions to the amino acid incorporating system using L-[U-^{14}C]leucyl-tRNA. Controls received 0.01 ml of the elution buffer which caused a 20% inhibition probably resulting from the presence of Ni^{2+}; amino acid incorporation obtained under the latter condition was taken as 100%. As shown in Figure 3, eluate corresponding to fraction I did not affect amino acid incorporation. Neither did commercially available GSSG in amounts up to 0.5 μmol, which was considerably higher than that which could arise from the inhibitory combination of 25 nmol of sodium selenite and 0.1 μmol of glutathione. This finding is in accord with the lack of inhibition by GSSG on amino acid incorporation by polyribosomes freed from membrane by Triton X-100, in contrast to the inhibition exerted by GSSG when unfractionated microsomes were used (Nolan and Hoagland, 1971).

Eluate corresponding to fraction II markedly inhibited amino acid incorporation (Figure 3). The pooled fraction II was subsequently concentrated by absorption to a column of Dowex 50W-X4, and eluted with 0.1 M ammonium acetate buffer, pH 5.5, according to Ganther (1971). Since lyophilization of the solution failed in our case due to decomposition yielding a reddish precipitate, the concentrate was further purified on Sephadex G-10 (2.5 × 35 cm) made up and eluted with twice distilled water. The uv absorption spectrum of the

TABLE IV: Inhibitory Effect of the Reaction Product of Sodium Selenite and Glutathione on Leucine Incorporation from L-[U-^{14}C]Leucyl-tRNA into Protein by Free Polyribosomes.[a]

Sephadex G-10 Eluate (ml)[b]	cpm	% Inhibition
	2234	
0.01	2092	6
0.02	870	61
0.04	111	95
0.06	64	97
0.08	60	97
0.10	67	97
0.20	42	98

[a] Final volume 1 ml. [b] The absorbance of the Sephadex G-10 eluate at 263 nm amounted to 1.0. For details about the chromatography with Dowex 50W-X4 and the subsequent gel filtration with Sephadex G-10 see text.

eluate was again similar to that assigned by Ganther (1971) to GSSeSG. The eluate remained stable for several weeks at 4° after which time decomposition became apparent from the gradual disappearance of the maximum (263 nm) and minimum (252 nm) in the uv absorption spectrum.

The fresh eluate was next tested for its inhibitory effect on the incorporation of leucine from L-[U-^{14}C]leucyl-tRNA into protein (Table IV). An amount corresponding to 0.02 absorption unit at 263 nm exerted already 60% inhibition whereas maximum inhibition (≥95%) resulted from ≥0.04 absorption unit. Taking into account the millimolar extinction coefficient of 1.87 at 263 nm for GSSeSG (Ganther, 1971), the dose–response curve of Table IV closely resembled that obtained with Na_2SeO_3 in Figure 1.

Finally, 0.10 absorption unit produced 100% inhibition of DL-[1-^{14}C]leucine incorporation into the protein made by free polyribosomes supplemented with either the 150,000*g* supernatant or the Sephadex G-25 fractions 15–17 derived therefrom. This amount inhibited L-[U-^{14}C]leucyl-tRNA formation for 31%.

Discussion

"The fate of selenious acid, or selenite, in systems containing thiols is relevant to many problems regarding the nutritional and toxicological aspects of this substance" (Ganther, 1971).

In the present study sodium selenite has been shown to be a very potent inhibitor of polyribosomal amino acid incorporation subsequent to the formation of aminoacyl-tRNA, outranking cycloheximide in its quantitative effect. In order to be active as such selenite required the presence of another low molecular weight factor, and our results make it likely that this factor was a thiol endogenous to liver, very probably also glutathione. A reaction product between glutathione and sodium selenite was likewise inhibiting. This product could be the selenotrisulfide derivative of glutathione (GSSeSG) according to Ganther (1971). However, since GSSeSG has not been characterized fully and the compound is relatively unstable, it cannot be excluded that another reaction product or a degradation product is the ultimate inhibitor. Work is in progress along these lines.

The aminoacyl-tRNA synthetases and the transferases are SH-dependent enzymes (Jocelyn, 1972b; Sutter and Moldave,

1966). Reduced SH groups are also required on the ribosome for allocating transferase II (Baliga and Munro, 1971). It has been shown in Table III that sodium selenite does not react in an inhibitory fashion with the SH groups of transferases and ribosomes. This is understandable since the stoichiometry of the reaction requires four SH groups per molecule of Na_2SeO_3 and such a reaction with protein–SH groups would be highly unlikely for steric reasons.

In view of the high potency of the inhibitory compound formed between Na_2SeO_3 and glutathione on amino acid incorporation, it is suggested that the latter effect may contribute to the known toxicity of selenite.

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References

Baliga, B. S., and Munro, H. N. (1971), *Nature (London), New Biol. 233*, 257.

Bloemendal, H., Bont, W. S., De Vries, M., and Benedetti, E. L. (1967), *Biochem. J. 103*, 177.

Bloemendal, H., Huizinga, F., De Vries, M., and Bosch, L. (1962), *Biochim. Biophys. Acta 61*, 209.

Ganther, H. E. (1968), *Biochemistry 7*, 2898.

Ganther, H. E. (1971), *Biochemistry 10*, 4089.

Gasior, E., and Moldave, K. (1965), *J. Biol. Chem. 240*, 3346.

Harr, J. R., and Muth, O. H. (1972), *Clin. Toxicol. 5*, 175.

Jocelyn, P. C. (1972a), Biochemistry of the SH Group, London, Academic Press, p 261.

Jocelyn, P. C. (1972b), Biochemistry of the SH Group, London, Academic Press, p 233.

Jose, P. J., and Slater, T. F. (1970), *Biochem. J. 117*, 66p.

Kirby, K. S. (1965), *Biochem. J. 96*, 266.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.

Nolan, R. D., and Hoagland, M. B. (1971), *Biochim. Biophys. Acta 247*, 609.

Rao, K. S., and Recknagel, R. O. (1968), *Exp. Mol. Pathol. 9*, 271.

Recknagel, R. O. (1967), *Pharmacol. Rev. 19*, 145.

Rosenfeld, I., and Beath, O. A. (1964), in Selenium, New York, N. Y., Academic Press, p 170.

Sandholm, M., and Sipponen, P. (1973), *Arch. Biochem. Biophys. 155*, 120.

Shapiro, J. R. (1972), *Ann. N. Y. Acad. Sci. 192*, 215.

Sutter, R. P., and Moldave, K. (1966), *J. Biol. Chem. 241*, 1698.

Tappel, A. L. (1965), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 24*, 73.

Vernie, L. N., Bont, W. S., and Emmelot, P. (1971), *Cancer Res. 31*, 2189.

Early Effect of Estradiol on the Peptide Elongation Rate by Uterine Ribosomes†

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ABSTRACT: A cell-free protein synthesis system employing uterine ribosomes was developed. The *rate* of protein synthesis ([^{14}C]leucine incorporation/10 min per 100 μg of rRNA) and the *number* of active ribosomes ([^{3}H]puromycin-peptide formed/100 μg of rRNA) were determined. The *rate* of protein synthesis by uterine ribosomes from ovariectomized mature rats increases biphasically with time after injection of estradiol *in vivo* with a maximum at 1 hr after hormone treatment which is 268% of control values. The *number* of active ribosomes increased asymptotically to a level at 12 hr which was 210% of control values. The increase in *number* of active ribosomes closely parallels the increase in *rate* of protein synthesis at all times after estradiol administration except 1 hr at which time the increase in *rate* of protein synthesis could not be accounted for by the increase in *number* of growing peptides in the ribosome preparation. The early effect of estradiol at 1 hr on protein synthesis appears to result from an increased rate of peptide elongation on each active ribosome. Uterine ribosomes from 1-hr estradiol-treated animals exhibit a greater dependency on the addition of GTP to the protein synthesis system than those from control or 4- and 12-hr estradiol-treated animals. Also, the nascent peptides released from ribosomes of 1-hr estradiol-treated animals by [^{3}H]puromycin are of a greater length than those released from control ribosomes suggesting that the *in vivo* rate of elongation has increased relative to the rate of initiation of synthesis of new peptides. The early increase in peptide elongation rate is not inhibited by actinomycin D.

Estradiol causes an increase in the rate of synthesis of protein in the uterus of the immature or ovariectomized mature rat. This effect can be seen when protein synthesis is measured in various *in vivo* as well as *in vitro* systems ranging from uterine tissue slices to isolated uterine ribosomes (Ui and Mueller, 1963; Noteboom and Gorski, 1963; Hamilton, 1964; Means and Hamilton, 1966a; Greenman and Kenney, 1964; Teng and Hamilton, 1967a,b; Suvatte and Hagerman, 1970). Most of these studies suggest that the estrogen effect on protein

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