EVIDENCE FOR REGULATION OF PROTEIN SYNTHESIS AT THE TRANSLATION LEVEL IN RESPONSE TO DIETARY ALTERATIONS

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ABSTRACT

Rats were maintained for several days on a protein-free diet which, approximately 14 hr before decapitation, was changed to a protein-rich diet. Microsomal subfractions of liver were obtained by sucrose density gradient centrifugation. The sedimentation patterns of the microsomal subfractions and of the polysomes were found to be unchanged after the alteration of the diet whereas the ability to incorporate amino acid was markedly enhanced. No significant differences in the RNA/protein ratio of the microsomal subfractions were observed. The changes in amino acid incorporation which were unrelated to concurrent changes in the amount of polysomes are discussed.

INTRODUCTION

It is known that certain tissue proteins rapidly increase or diminish under the influence of nutritional alterations, and general aspects of this subject have recently been reviewed (14). The changes in protein metabolism may be reflected in a very direct way in the protein-synthesizing capacity of the endoplasmic reticulum. It is generally accepted that protein synthesis is regulated at the level of gene transcription (6). Thus, the rate of synthesis is controlled by the rate of formation of messenger RNA. Regulation of protein synthesis at the translation level has been suggested, however, and several reports provide evidence of alterations in polysome activities in the absence of a concomitant messenger RNA synthesis (5, 7, 11).

Dietary carbohydrates exert an influence on the protein content of liver, and an increase in protein has been shown in the liver of rats receiving a protein-free diet and then an isocaloric diet rich in protein (14). In the present study, an attempt was made to apply these dietary conditions to elucidating the ability of the endoplasmic reticulum of rat liver to respond to such environmental changes. A method for subfractionating microsomes from liver was used so that we could obtain a differentiation of the microsomes into fractions with high and low amino acid–incorporating activities. The changes obtained in the activities of the microsomal subfractions could not be related to a dissociation or reformation of polysomes. The data are interpreted to suggest a transient regulation of protein synthesis by factors other than messenger RNA but possibly associated with the polysomes themselves.

A preliminary report of this work has been published (4).

EXPERIMENTAL

Materials

L-leucine-^{14}C, UL (Specific activity, 200 mc/mmole), and DL-leucine-1-^{14}C (20 mc/mmole) were obtained from New England Nuclear Corp., Boston. ATP (disodium salt), GTP (sodium salt), Tris-HCl, Tris base, and bovine serum albumin were obtained from Sigma Chemical Co., St. Louis. Cadmium-free

horse spleen ferritin was obtained from Pentex Inc., Kankakee, Ill.; pyruvate kinase from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany, and sodium deoxycholate and yeast RNA from E. Merck A.-G., Darmstadt, Germany. PEP[1] was synthesized as described by Clark and Kirby (2), and was recrystallized three times as its monocyclohexylammonium salt. Before use, the salt was dissolved in 0.2 M Tris-HCl buffer, pH 7.8 (0.5 ml/30 mg of salt), and adjusted to pH 7 with 0.7 N KOH. All solutions were made up in glass-distilled water. The media used for sucrose gradient centrifugation were autoclaved so that we could reduce contamination by RNase. The pH values were obtained at 25°. The preparative procedures were carried out at 0–4°.

Animals

Sprague-Dawley female rats (130–180 g) were starved overnight. They were then kept for 2 days on a carbohydrate-containing diet consisting of 45% glucose, 45% potato starch, and 10% vegetable fat. On the 3rd day, one control group was maintained on the same diet, while another group received a protein-rich diet consisting of 50% casein, 20% glucose, 20% potato starch and 10% vegetable fat. The energy intake per rat was about 1000 kcal/m^2/day. These diets were essentially those described by Munro and Naismith (15). The meals were given at about 7 PM and the rats were killed at 9 AM the following morning by a blow on the head and decapitation. The liver was perfused with ice-cold 0.15 M KCl and then placed into medium A (35 mM Tris buffer, pH 7.8, 25 mM KCl, 0.05 M NH_4Cl, 0.01 M $MgCl_2$, and 0.25 M sucrose). The minced liver was homogenized in 10–12 ml of medium A. The homogenate was centrifuged in an International High-Speed Refrigerated Centrifuge, Model H-R-1, rotor No. 856. Centrifugation was for 70,000 *g*-min (5 min at 14,000 *g*). The supernatant obtained is called mitochondrial supernatant.

Microsomes and Cell Sap

The total microsomal fraction and cell sap were obtained by our centrifuging the mitochondrial supernatant for 70 min at 165,000 *g* in a Spinco model L preparative centrifuge (No. 50 rotor). Only the clear supernatant was used as cell sap. The microsomal pellet was suspended and gently homogenized in medium A so that it would give a final protein concentration of about 12 mg/ml.

[1] The following abbreviations are used: PEP, phosphoenolpyruvate. PCA, perchloric acid. PPO, 2,5-diphenyloxazole. Dimethyl-POPOP dimethyl-1,4-bis-2-(4-methyl-5-phenyloxazolyl)-benzene.

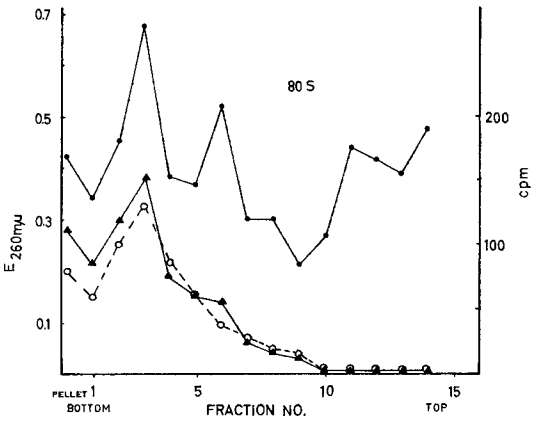

FIGURE 1 Amino acid-incorporating activity of microsomal subfractions from normal rats prepared by discontinuous sucrose gradient centrifugation. Part of the fractions was used directly in amino acid incorporation experiments while the remainder was diluted with medium A and centrifuged for 1 hr at 165,000 *g*. The pellets were resuspended in the original volumes and their amino acid-incorporating activity was determined. The optical density (●——●) was measured after diluting 30 μl of the original fractions or of the reisolated, resuspended fractions to 1 ml. The radioactivity data correspond to the incorporation obtained in 30 μl of the undiluted fractions. Radioactivity of original fractions, ▲——▲; radioactivity of reisolated, resuspended fractions, O - - - O.

The incubation system contained in a final volume of 130 μl the gradient fraction (between 0.05 and 0.08 mg of protein for fraction 1-10), cell sap (0.5 mg of protein), 1 mM ATP, 0.2 mM GTP, 10 mM PEP, 40 μg/ml pyruvate kinase, about 5.5 mM $MgCl_2$, 25 mM KCl, 0.05 M NH_4Cl, 35 mM Tris-HCl buffer pH 7.8, and 0.01 mM labeled leucine (20 mc/mmole). After incubation for 15 min at 35°, 90-μl samples of the incubation mixture were transferred to filter paper discs and the proteins were processed as described under Experimental.

Sucrose Density Gradient Centrifugation

DISCONTINUOUS GRADIENTS: Solutions of 50, 40, 30, 25% (w/w) of sucrose were prepared in medium A containing 8 mM $MgCl_2$. (The final concentration of $MgCl_2$ was thus between 5 and 6.5 mM dependent on the sucrose concentration used). Volumes of 0.9, 1.2, 1.0, and 0.9 ml, respectively, were layered into tubes of the SW 39 rotor of the Spinco model L centrifuge. The tubes were filled with mitochondrial supernatant and centrifuged for 105 min at 39,000 rpm (125,000 *g*) without braking. Horse ferritin was run in parallel so that the position of free ribosomes in the gradient could be located (12). After centrifugation, we collected

fractions of 5–7 drops from the bottom of the gradient by puncturing the tube with a hypodermic needle. A small amount of each fraction was diluted with distilled water and the extinction determined in a Beckman model DB spectrophotometer at wavelengths of 240, 260, and 280 mμ. The optical density pattern obtained together with the amino acid–incorporating activity of the fractions are shown in Fig. 1. The optical density values were obtained after we diluted 30 μl of the original fractions to 1 ml. The amino acid–incorporating activities are expressed as cpm/30 μl of the undiluted fractions. The radioactivity was highest near the bottom of the gradient. A parallel run of horse ferritin indicated the approximate position of free ribosomes. These showed negligible activities (tube Nos. 8–10).

Since variations in the sucrose concentrations might affect the incorporation, parallel experiments were carried out in which each fraction was diluted with medium A and centrifuged for 1 hr at 165,000 *g*. The final pellets were resuspended in medium A, diluted to the original volume, and tested for their ability to incorporate labeled amino acids into protein. As shown in Fig. 1, the high sucrose concentrations of the original fractions did not inhibit the incorporation. A slight loss of activity was sometimes observed on reisolation of the fractions. Some of the UV-absorbing material in the fractions was lost during recentrifugation. Therefore, the data shown in Fig. 1 were recalculated to the optical density values obtained when 30 μl of the original fractions were diluted to 1 ml. Since the variation in sucrose concentration did not significantly influence the incorporation, the fractions collected after density gradient centrifugation were used directly in the amino acid incorporation experiments to be described.

LINEAR GRADIENTS: The sucrose-salt media were layered as above, but with 20 instead of 25% sucrose. The gradient was allowed to equilibrate overnight in the cold room. The linearity was tested by the measurement spectrophotometrically of the distribution of methylene blue added to the sucrose solutions at concentrations of 0.5, 0.4, 0.3, and 0.2%, respectively.

Amino Acid Incorporation in Vitro

The activity of amino acid incorporation was determined on 60-μl samples of the gradient fractions which contained 0.04–0.08 mg of protein (fractions 1–10) or 0.25–0.4 mg of protein (fractions 11–16). Each fraction was combined with 0.5 mg cell sap protein. The incubation medium contained 1 mM ATP, 0.2 mM GTP, 10 mM PEP, 40 μg/ml pyruvate kinase, about 5.5 mM $MgCl_2$, 25 mM KCl, 0.05 M

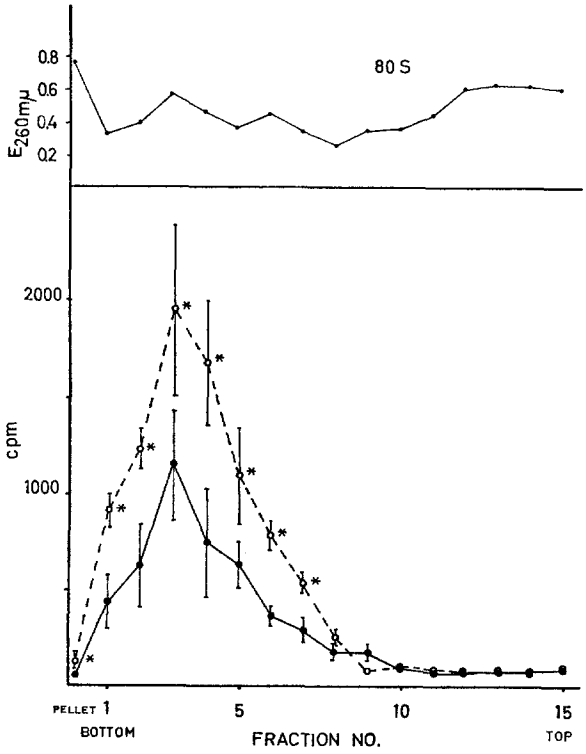

FIGURE 2 Effect of diet on the incorporation of leucine-^{14}C into protein by microsomal subfractions. Rats fed carbohydrates, ●——●; rats fed protein-rich diet, ○---○; optical density at 260 mμ, ●——●. The optical densities refer to both types of animals. The absorption was measured after diluting 30 μl of the gradient fractions to 1 ml. The radioactivity values correspond to the incorporation obtained in 30 μl of the undiluted fractions. The vertical lines indicate one standard deviation from the mean. Points statistically different from the values of the carbohydrate-fed animals are indicated by asterisks (*). Values are considered statistically different if the *P*-value is less than 0.05. The incubation system containing 0.0075 mM leucine-^{14}C (200 mc/mmole) was as described in Fig. 1.

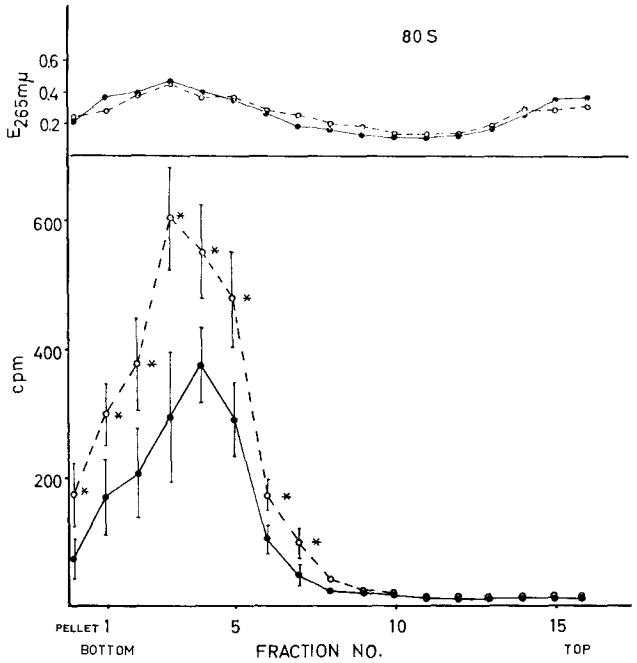

FIGURE 3 Effect of diet on the incorporation of leucine-^{14}C into protein by polysomes obtained from a mitochondrial supernatant of rat liver. Polysomes were obtained by treating the mitochondrial supernatant with 1% sodium deoxycholate. The suspension was layered on a linear sucrose density gradient (50–20%) and centrifuged for 105 min at 125,000 *g*. The incubation system containing 0.0035 mM leucine-^{14}C was as described in Fig. 1. The absorption was measured after precipitation of 30 μl of the gradient fractions with 1 ml of 0.2 N PCA and extraction of the RNA at 70°. The radioactivity values correspond to the incorporation obtained in 30 μl of the undiluted fractions. Rats fed carbohydrates, ●——●; rats fed protein-rich diet, O - - - O. The upper part of the curve shows the optical density values at 265 mμ. The vertical lines indicate one standard deviation from the mean. Points statistically different from the values of the carbohydrate-fed animals are indicated by asterisks (*). Values are considered statistically different if the *P*-value is less than 0.05.

NH_4Cl, 35 mM Tris-HCl buffer pH 7.8, and labeled leucine as indicated in the figures. The final volume was 130 μl. After incubation for 15 min at 35°, 90-μl samples were transferred to filter paper discs, and the proteins were processed by the method of Mans and Novelli (9). Radioactivity was measured at 40% efficiency in a Packard Tricarb liquid scintillation counter, with a toluene scintillation fluid containing 0.5% PPO and 0.015% POPOP.

Analyses

Protein was determined by the method of Lowry et al. (8) with bovine serum albumin as a standard. RNA was determined by the orcinol method of Mejbaum (13) with hydrolyzed yeast RNA as a standard. The amino acid content was estimated by the ninhydrin reaction according to Cocking and Yemm (3), leucine being used as a standard. Since the Tris-HCl buffer interferes with the determination, the cell-free preparations were made in 0.15 M KCl.

RESULTS

Amino Acid-Incorporating Activity of Microsomal and Polysomal Subfractions

Microsomes from rats maintained on a carbohydrate diet and fed, during the last 14 hr, either the same diet or an isocaloric, protein-rich diet were compared by discontinuous density gradient centrifugation. As shown in Fig. 2, the distribution of UV-absorbing material was about similar in the two preparations. However, the amino acid–incorporating activity of the particles was markedly enhanced in the case of the protein-fed rats. The difference was occasionally as great as

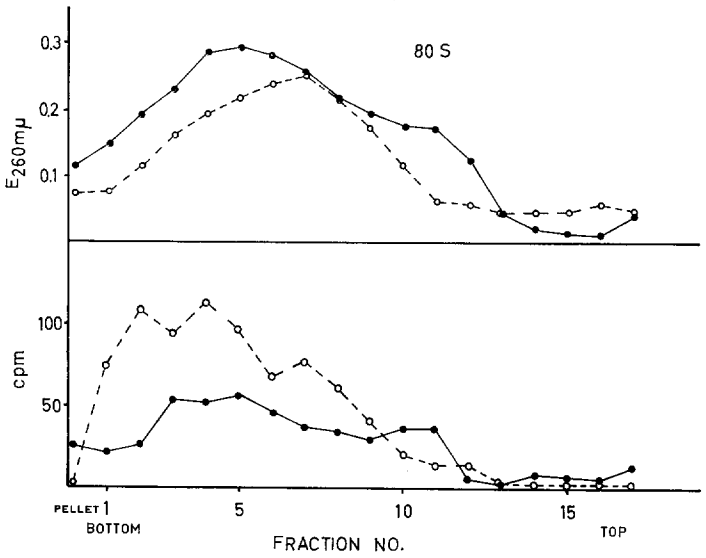

FIGURE 4 Effect of diet on the incorporation of leucine-^{14}C into protein by polysomes isolated from one microsomal subfraction. Optical density values are shown in the upper part of the figure. Polysomes were prepared by treating a microsomal subfraction (corresponding to tube No. 3 in Fig. 2) with 1% sodium deoxycholate and centrifuging the suspension through a linear sucrose density gradient (50–20% sucrose). Incubation (but with 0.001 mM leucine-^{14}C (200 mc/mmole)) was as described in Fig. 1. The absorption was measured after diluting 0.2 ml of the gradient fractions to 1 ml. The radioactivity values correspond to the incorporation obtained in 0.2 ml of the undiluted fractions. Rats fed carbohydrates, ●——●; rats fed protein-rich diet, O - - - O.

400–600% owing to a conspicuous drop in the activity of the microsomal subfractions of the carbohydrate-fed rats. These extreme values have not been included in the calculations shown in Fig. 2, since the exact conditions in vivo under which they were obtained have not yet been established.

Treatment of the mitochondrial supernatant with deoxycholate followed by linear density gradient centrifugation of the suspension revealed a rather similar polysomal pattern for the two types of rats (Fig. 3). This was also the case when we extracted subfractions obtained with PCA in order to remove the glycogen. Although the gradient sedimentation pattern of the polysomes was similar, the ability to incorporate amino acids was greatly enhanced after the feeding of the proteins.

The heavy microsomal fraction showing a high amino acid–incorporating activity (fraction No. 3 in Fig. 2) was treated with sodium deoxycholate and recentrifuged through a linear sucrose gradient. As shown in Fig. 4, the optical density pattern indicated the presence of polysomes, and this was confirmed by the high incorporating activity of the fractions. A comparison of the fractions obtained from carbohydrate-fed rats with those from protein-fed rats showed only a slight difference in the optical density pattern, whereas the amino acid–incorporating activity was significantly higher in the fractions obtained from protein-fed rats.

The results presented in Figs. 2–4 clearly indicate that, under the conditions described, the nutritional alterations seemed to have but little effect on the proportion of microsomes and polysomes present in liver. On the other hand, it is shown that the ability to incorporate amino acids as measured by UV-absorbing material was subject to markedly significant changes.

Rate of Amino Acid Incorporation

The time course of amino acid incorporation by unfractionated microsomes is illustrated by Fig. 5. The increase in activity after protein administration was already evident at short-time incubations. The lifetime of the microsomal preparations appeared not to be influenced by the nutritional conditions. Furthermore, the lower activity of the

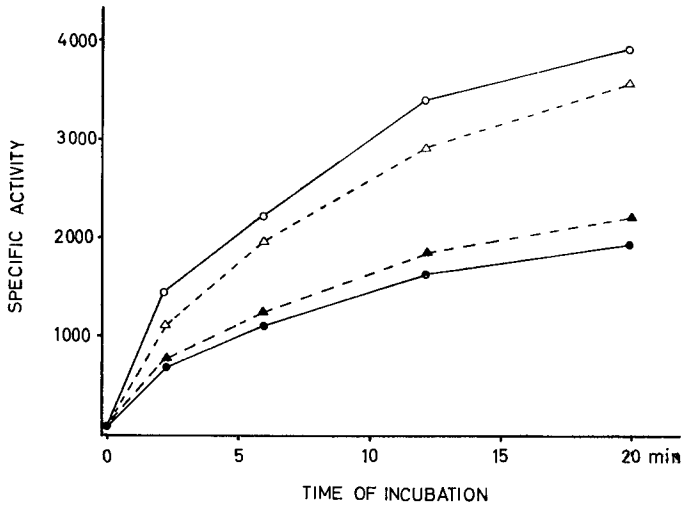

FIGURE 5 Effect of diet on the rate of amino acid incorporation by total microsomes. The incubation system was that described in Fig. 1, but combining total microsomes and cell sap as indicated. The same amounts of microsomal protein (0.7 mg) were added in all cases. As labeled amino acid, 0.025 mM leucine-^{14}C (20 mc/mmole) was used. ●——●, microsomes and cell sap from carbohydrate fed rats; ▲---▲, microsomes from carbohydrate-fed rats and cell sap from protein-fed rats; ○——○, microsomes and cell sap from protein-fed rats; △---△, microsomes from protein-fed rats and cell sap from carbohydrate-fed rats. The specific activity is expressed as cpm/mg microsomal protein. The RNA/protein ratios of the microsomes were 0.21 in the case of carbohydrate-fed rats and 0.22 in the case of protein-fed rats.

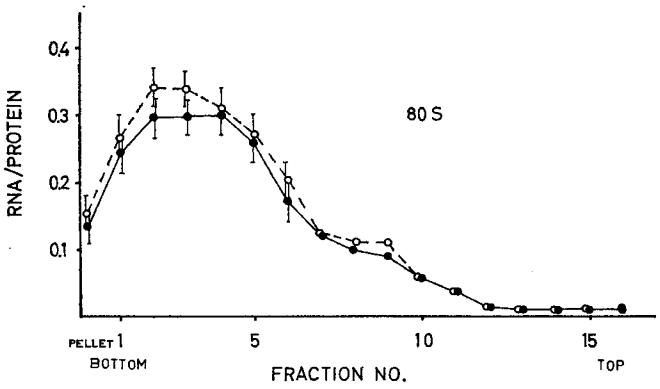

FIGURE 6 RNA/protein ratio of microsomal subfractions from carbohydrate-fed rats (●——●) and from protein-fed rats (○---○). The vertical lines indicate one standard deviation from the mean.

microsomes of the carbohydrate-fed rats was a property of the microsomes or ribosomes themselves rather than of the cell sap. Incubation of the microsomes from carbohydrate-fed rats with cell sap from protein-fed animals did not enhance the incorporation activity of the particles to a level comparable to that of microsomes from protein-fed rats. The amount of free amino acids in the cell sap showed no significant difference between the two types of rats. The content was estimated to about 0.025 mg/ml cell sap protein.

RNA content

As shown in Fig. 6, the difference in the activity of the microsomes in amino acid incorporation could not be related to a difference in the RNA/protein ratios of the microsomal subfractions. In both types of rats, the heavy fractions contained a high proportion of the ribosomal RNA which gradually decreased towards the top of the gradient.

DISCUSSION

Polysomes are not dissociated into ribosomes when rats are fed a carbohydrate-containing diet depleted in protein. These findings are compatible with the results of Kerr et al. (7) which indicate that the presence of glucose is sufficient to prevent polysomal disaggregation in Krebs II mouse ascites tumor cells. Thus, the supply of energy seems to be a basic factor influencing the formation and the stabilization of polysomes (1, 16). In intact reticulocytes a dissociation of polysomes is produced by NaF, a strong inhibitor of glucose metabolism (11). A considerable dissociation of polysomal aggregates into monoribosomes was also obtained when rats were kept for several days on a protein-depleted diet and then starved for 18 hr (10). Under those conditions, the amino acid–incorporating activity decreased simultaneously.

The experiments described here were carried out with preparations obtained from nonstarved animals. In spite of the fact that, under those conditions, the proportion of polysomes remained constant, remarkable alterations in the activity of protein synthesis were demonstrated. These changes were entirely dependent on the previous nutritional conditions. The variation in the amino acid–incorporating activity cannot be attributed to a variation in the messenger RNA content of the system. The amount of free ribosomes was much too small to account for the striking increase in activity by attachment of these ribosomes to new messenger RNA. It seems more likely that the polysomes from the different kinds of preparations had different read-out velocities.

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REFERENCES

1. BAGLIO, C. M., and E. FARBER. 1965. *J. Mol. Biol.* **12**:466.
2. CLARK, V. M., and A. J. KIRBY. 1963. *Biochim. Biophys. Acta.* **78**:732.
3. COCKING, E. C., and E. W. YEMM. 1954. *Biochem. J.* **58**:Xii.
4. DECKEN, A. VON DER. 1966. Abstracts of the 3rd Meeting of the Federation of European Biochemical Societies, Warsaw. Academic Press Inc., New York. 201.
5. HOAGLAND, M. B., O. A. SCORNIK, and L. C. PFEFFERKORN. 1964. *Proc. Natl. Acad. Sci.* **51**:1184.
6. JACOB, F., and J. MONOD. 1961. *J. Mol. Biol.* **3**:318.
7. KERR, I. M., N. COHEN, and T. S. WORK. 1966. *Biochem. J.* **98**:826.
8. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, and R. J. RANDALL. 1951. *J. Biol. Chem.* **193**:265.
9. MANS, R. J., and G. D. NOVELLI. 1961. *Arch. Biochem. Biophys.* **94**:48.
10. MANDEL, O., C. QUIRIN, M. BLOCH, and M. JACOB. 1966. *Life Science.* **5**:325.
11. MARKS, O. A., E. R. BURKA, F. M. CONCONI, W. PERL, and R. A. RIFKIND. 1965. *Proc. Natl. Acad. Sci. U. S.* **53**:1437.
12. MARTIN, R. G., and B. H. AMES. 1961. *J. Biol. Chem.* **236**:1372.
13. MEJBAUM, W. 1939. *Hoppe Seyler's Z. Physiol. Chem.* **258**:117.
14. MUNRO, H. N. 1964. *In* Mammalian Protein Metabolism. H. N. Munro and J. B. Allison, editors. Academic Press Inc., New York. **1**:381.
15. MUNRO, H. N., and D. J. NAISMITH. 1953. *Biochem. J.* **54**:191.
16. WEBB, T. E., G. BLOBEL, and R. VAN POTTER. 1966. *Cancer Res.* **26**:253.