Biochemical Pharmacology, Vol. 24, pp. 1019–1023. Pergamon Press, 1975. Printed in Great Britain.

INHIBITED INITIAL RATES OF POLY-URIDYLIC ACID-DIRECTED PHENYLALANINE INCORPORATION BY FREE RIBOSOMES FROM THE LIVER OF RATS FED HEPATOCARCINOGENS*

DONALD E. KIZER and JOSEPH A. CLOUSE

Biomedical Division, The Samuel Roberts Noble Foundation, Inc., Ardmore, Okla. 73401, U.S.A.

(*Received* 25 *May* 1974; *accepted* 11 *October* 1974)

Abstract—Female Holtzman rats were fed diets containing 3′-methyl-4-dimethyl-aminoazobenzene (3′-Me-DAB) for 4 weeks; control rats were fed the basal diet. Free ribosomes and membrane-bound ribosomes were isolated from livers and initial rates of phenylalanine incorporation *in vitro* into proteins were determined both in the absence and presence of poly-uridylic acid (poly-U). Initial rates of protein synthesis by membrane-bound ribosomes were not detectably affected by 3′-Me-DAB ingestion. Although initial rates for free ribosomes from control rat livers were stimulated by poly-U additions, rates for free ribosomes from 3′-Me-DAB-fed rats were not appreciably changed by poly-U. Defective poly-U-directed phenylalanine incorporation by free ribosomes from rats fed 3′-Me-DAB: (a) was dependent upon time-on-diets; (b) was not an artifact associated with the method of isolating free ribosomes; (c) was not corrected by changing the magnesium level of reaction mixtures or by supplementing the levels of pH 5 precipitate fraction, elongation factors or initiation factors; and (d) was not due to a failure to bind poly-U. Feeding other hepatocarcinogens, viz. 4′-fluoro-DAB, 2-acetylaminofluorene, aflatoxin and thioacetamide, to female Holtzman rats or feeding any of these five hepatocarcinogens to male Fischer rats also led to the development of defective poly-U-directed phenylalanine incorporation by hepatic free ribosomes. Male Holtzman rats and female Fischer rats developed the defect more slowly, i.e. 8 weeks of feeding was necessary before the defect was detected with three of the five hepatocarcinogens. The possibility that defective poly-U-directed phenylalanine incorporation was due to a ribosomal alteration, and the possibility of a linkage between this phenomenon and the animal's risk for cancer were discussed.

Feeding rats hepatocarcinogens results in both biochemical and cytological changes in the liver. An early prominent biochemical change is the covalent binding of hepatocarcinogen metabolites to nucleic acids and protein [1–6]. An early prominent cytological change is the loss of rough endoplasmic reticulum and increases in the amount of smooth endoplasmic reticulum and free ribosomes [7–12].

A substantial portion of the translational system of rat liver is firmly bound to the endoplasmic reticulum [13, 14]. The remainder occurs free or loosely attached to membranes [15, 16]. Membrane-associated ribosomes mainly synthesize exportable proteins [17–28], whereas free ribosomes mainly synthesize non-exportable proteins [23, 25, 29, 30].

The translational systems of rat liver are affected by hepatocarcinogens. For instance, protein synthesis both *in vivo* and *in vitro* was affected by the administration of carcinogenic chemicals [2, 8, 31–45]. Studies with DMN** showed this inhibition to be accompanied by breakdown of polyribosomes to monosomes [40], and Vernie *et al.* [44] attributed inhibition of protein synthesis *in vitro* to this accumulation of monosomes. On the other hand, Hultin [40] attributed inhibition primarily to a disturbed peptide chain initiation. In a recent study, some subunits, from the monoribosome fraction isolated from the liver of DMN-injected rats, either failed to form 80S couples or to support poly-U-directed polyphenylalanine synthesis [45].

It was our purpose to determine whether hepatocarcinogen feeding to rats appreciably affected protein synthesis *in vitro* by free or membrane-associated hepatic ribosomes. Accordingly, hepatocarcinogens were fed to rats and initial rates of phenylalanine incorporation into protein were determined in the presence and absence of poly-U. With membrane-bound ribosomes, this rate was unaffected by hepatocarcinogen feeding whether poly-U was present or absent. With free ribosomes, this rate was unaffected by hepatocarcinogens in the absence of poly-U, but in the presence of poly-U initial rates were inhibited.

MATERIALS AND METHODS

Animals. Unless otherwise indicated, female Holtzman rats were used in these experiments. In one series of experiments, male Holtzmann rats and both male and female Fischer rats were used. Holtzman rats were purchased, but Fischer rats were born and reared in our own breeding colony. Rats usually

* Portions of the data contained herein were presented at a symposium on "Recent Advances in the Biochemical Pathology of Disease," the proceedings of which were published in *Ann. Okla. Acad. Sci.* **4**, 11 (1974).

** The following abbreviations are used: DMN, dimethylnitrosamine; poly-U, poly-uridylic acid; DAB, 4-dimethylaminoazobenzene; 2-AAF, 2-acetylaminofluorene; α-NIT, α-naphthylisothiocyanate; STKM-buffer, 0·25 M sucrose, 0·05 M Tris, pH 7·4, 0·025 M KCl and 0·005 M $MgCl_2$; rRNA, ribosomal RNA.

were committed to an experimental protocol at about 12 weeks of age. They were fed either Teklad Mouse and Rat Diet or a semi-synthetic diet [46]. The hepatocarcinogens were added to the semi-synthetic diet after solubilization in appropriate solvent, i.e. 3′-methyl-DAB (3′-Me-DAB) and 4′-fluoro-DAB (4′-F-DAB) were dissolved in corn oil and both were added to give a concentration of 0·06% by weight, 2-AAF and thioacetamide were dissolved in ethanol and were added to give concentrations of 0·02 and 0·07%, respectively, and aflatoxin was dissolved in acetone and was added to give a concentration of 4 mg/kg of diet. Two hepatotoxic agents also were fed to rats. These were 4′-methyl-DAB (4′-Me-DAB) which was dissolved in corn oil and fed at 0·06% and α-NIT which was dissolved in ethanol and fed at 0·04%. In all experiments, rats were fasted 24 hr prior to killing by cervical dislocation.

Protein synthesis in vitro. Free or membrane-bound ribosomes used in most experiments were isolated from homogenates of rat liver employing a procedure which was described by Hallinan and Munro [47], but in several experiments, a procedure described by Blobel and Potter [48] was used. Deoxycholate was used to release membrane-bound ribosomes. Release was accomplished by resuspending microsome fractions in STKM-buffer [49] containing 1% deoxycholate. After release, ribosomes were recovered by centrifugation for 1 hr at 105,000 *g*. Ribosomes isolated from the liver of rats fed basal or carcinogen-containing diets were added to reaction mixtures for protein synthesis *in vitro* in amounts equivalent to 300 ± 30 μg ribosomal RNA.

The reaction mixture for protein synthesis *in vitro* was essentially identical to that used by Tsukada *et al.* [50]. Sufficient L-phenylalanine-U-^{14}C (about 410 mCi/m-mole) was added to this reaction mixture to give a concentration of 0·04 mM and an isotope concentration of 0·2 μCi/ml. The total volume was 1 ml. Unless otherwise indicated, the incubation period was 5 min at 37°.

The pH 5 precipitate fraction for this reaction mixture was isolated from the liver of stock rats [51]. In several experiments, the reaction mixture was supplemented with partially purified elongation factors and initiation factors. Elongation factors 1 and 2 were purified from pH 5 supernatant fractions using the procedure of Moldave [52] except that final separation of the two factors by gel filtration was deleted. Elongation factor 2 activity of these preparations was estimated by following the hydrolysis of GTP-γ^{32}P to GDP and ^{32}P-inorganic phosphate [53] in the presence of ammonium chloride-washed ribosomes [54]. Photophosphorylation of GDP in the presence of ^{32}P-inorganic phosphate [53] was catalyzed by a crude preparation of spinach chloroplasts [55]. Initiation factors were isolated from rabbit reticulocyte ribosomes [56] using the procedure described by Miller and Schweet [57].

Protein synthesis *in vitro* was halted by addition of 5% trichloroacetic acid containing 0·2% unlabeled phenylalanine. Precipitated proteins were washed [58], solubilized in NaOH and reprecipitated with trichloroacetic acid. Radioactivity was estimated by liquid scintillation counting [59, 60] and radioactivity was expressed relative to the rRNA content which was estimated by reaction with orcinol [61]. In 14 experiments performed during data acquisition, ribosomes from rats fed basal diets polymerized phenylalanine in the presence of poly-U at rates ranging from 317 ± 34 to 1832 ± 101 pmoles/mg of rRNA/5-min incubation, while the range for ribosomes from 3′-Me-DAB-fed rats was 169 ± 14 to 1135 ± 81 pmoles/mg of rRNA/5 min.

The extent to which ^{3}H-poly-U was bound to hepatic ribosomes was determined essentially by the nitrocellulose membrane technique described by Moore [62]. Membranes were saturated with bovine serum albumin [62], and about 150 μg ribosomes was layered on each membrane [63]. Then 3 ml of a ^{3}H-poly-U solution (7 μg poly-U/ml containing about 600 dis/min/μg) was passed through each membrane. The control system consisted of membranes containing formaldehyde-treated ribosomes [62].

RESULTS

A consequence of feeding rats 3′-Me-DAB was the inhibition of initial rates of poly-U-directed polyphenylalanine synthesis by free hepatic ribosomes. Data illustrating this effect are shown in Fig. 1. After

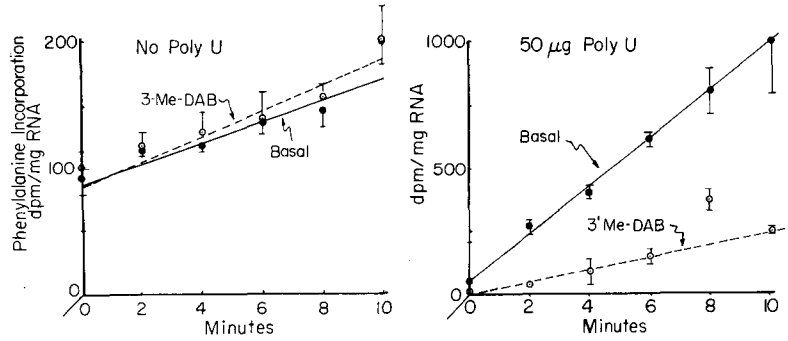

Fig. 1. Effect of poly-uridylic acid on the time-course incorporation of phenylalanine into protein by free ribosomes isolated from the liver of rats fed 3′-Me-DAB. Rats were fed basal diet or this diet containing 0·06% 3′-Me-DAB for 4 weeks. Free ribosomes were isolated [47], resuspended in STKM-buffer [49], and were added to reaction mixtures for protein synthesis *in vitro* [50]. Reaction mixtures also contained pH 5 precipitate fraction [51] and partially purified elongation factors 1 and 2 [52]. Where indicated, poly-U was present at 50 μg/reaction mixture. Values are averages ± standard errors for ten rats on each data point.

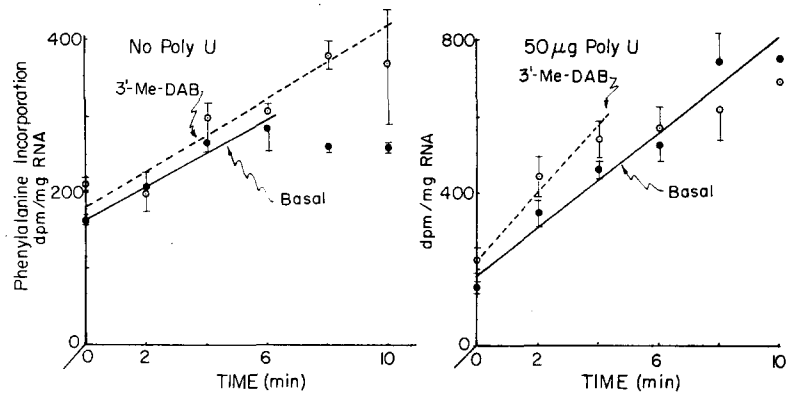

Fig. 2. Time-course incorporation of phenylalanine into protein by membrane-bound ribosomes isolated from the liver of rats fed 3'-Me-DAB. Microsomes were isolated [47] and membrane-bound ribosomes released using 1% deoxycholate. Other conditions were as described in Fig. 1 except that partially purified elongation factors were deleted from reaction mixtures. Values are averages ± standard errors for four rats on each data point.

the animals had been on diets for 4 weeks, free ribosomes were isolated [47]. In the absence of poly-U, ribosomes from basal- or 3'-Me-DAB-fed rats catalyzed phenylalanine incorporation with equal efficiency. Upon the addition of poly-U, however, phenylalanine incorporation by basal ribosomes was markedly stimulated, whereas ribosomes from 3'-Me-DAB-fed rats showed only moderate stimulation. When a similar experiment was performed using membrane-bound ribosomes, data as shown in Fig. 2 were obtained. Although incorporation was not always linear throughout 10 min, in both the presence and absence of poly-U, phenylalanine incorporation by either ribosomal population proceeded at essentially equal rates. Thus, inhibition of poly-U-directed phenylalanine incorporation seemed to be a property uniquely associated with the free ribosomal population from the liver of rats fed 3'-Me-DAB. Therefore, throughout the remainder of this paper, experiments involved only free ribosomes.

Since it was possible that exposure of ribosomes to iso-octane during their isolation [47] contributed to their limited responsiveness to poly-U (Fig. 1), free ribosomes were isolated by centrifugation through discontinuous sucrose gradients [48]. As shown in Table 1, after 4 weeks of feeding, ribosomes from the liver of basal- or 3'-Me-DAB-fed rats catalyzed poly-U-directed phenylalanine incorporation at essentially equal initial rates. Upon continuing the feeding period through 8 weeks, however, free ribosomes from the liver of 3'-Me-DAB-fed rats catalyzed poly-U-directed phenylalanine incorporation at significantly slower initial rates. Perhaps the failure to detect inhibition after 4 weeks of 3'-Me-DAB feeding was associated with losses of free monosomes in the overlying 2 M sucrose layer [64–66]; nevertheless, inhibition after 8 weeks of feeding argued against the possibility that inhibition depicted in Fig. 1 was due to some interaction between 3'-Me-DAB ribosomes and iso-octane.

Table 1. Effect of 3'-Me-DAB upon poly-uridylic acid-directed phenylalanine incorporation by hepatic free ribosomes which were isolated by centrifugation through discontinuous sucrose gradients*

Length of feeding period	Phenylalanine incorporation (dis/min/mg rRNA) Basal	3'-Me-DAB	(% of basal)†
4 Weeks			
Experiment 1‡	670 ± 20§	600 ± 40	90 ± 7
Experiment 2	650 ± 10	670 ± 10	103 ± 2
8 Weeks			
Experiment 1	760 ± 60	610 ± 30‖	80 ± 8
Experiment 2	1010 ± 50	860 ± 50‖	85 ± 7

* Ribosomes were isolated by the procedure of Blobel and Potter [48]. Other conditions were as described in Fig. 1 or in Methods. Incubation was for 5 min at 37°.

† Calculated by: $(\bar{X}_{3'\text{-Me}}/\bar{X}_{Basal})\ 100 \pm (\bar{X}_{3'\text{-Me}}/\bar{X}_{Basal})\ [(S.E._{3'\text{-Me}}/X_{3'\text{-Me}})^2 + (S.E._{Basal}/X_{Basal})^2]^{\frac{1}{2}}100$.

‡ The two experiments were spaced more than 6 months apart to remove possible bias introduced by a particular lot of animals.

§ Values are averages ± standard error (S.E.) of the mean for ribosomes from six rats.

‖ Values different from corresponding basal values at $P < 0.1$ or better.

Table 2. Effect of feeding hepatocarcinogens or hepatotoxic agents upon poly-uridylic acid-directed phenylalanine incorporation into protein by hepatic free ribosomes

Diets fed*	Phenylalanine incorporation† (dis/min/mg rRNA)	(% of basal)
Hepatocarcinogens		
Basal	470 ± 50	
Aflatoxin	350 ± 30‡	79 ± 11
4'-F-DAB	340 ± 40‡	72 ± 12
Thioacetamide	310 ± 30‡	66 ± 10
2-AAF	270 ± 40‡	58 ± 11
3'-Me-DAB	250 ± 20‡	53 ± 7
Hepatotoxic agents		
4'-Me-DAB	430 ± 30	92 ± 12
α-NIT	320 ± 20‡	68 ± 8

* Diets were fed to female Holtzman rats for 4 weeks. Poly-U was present at 50 μg/reaction mixture. Incubation time was 5 min. Other conditions were as described in Fig. 1.

† Specific activity values and per cent values were as described in Table 1. Each value was computed from data on ribosomes from seven to nine rats.

‡ Values different from basal values at $P < 0.1$ or better.

The development of inhibited initial rates of poly-U-directed phenylalanine incorporation was dependent upon the time 3′-Me-DAB was fed to rats. After 3 days, 1, 2 or 3 weeks, initial rates for ribosomes from 3′-Me-DAB-fed rats were 83 ± 7, 78 ± 6, 56 ± 4 or 62 ± 6% of rates determined for ribosomes from rats fed basal diet. The inhibition was reversible, for when 3′-ME-DAB was fed to rats for 4 weeks and then withdrawn from the diet, initial rates 3 weeks later were 96 ± 7% of rates determined for rats maintained on basal diets throughout 7 weeks.

Inhibited poly-U-directed phenylalanine incorporation by hepatic ribosomes from 3′-Me-DAB-fed rats could have been associated with correctable deficiencies in the reaction mixture for protein synthesis *in vitro*. However, lower initial rates persisted despite increases in the concentration of pH 5 precipitate fraction, magnesium, poly-U, elongation factors or initiation factors.

Inhibition was not associated with defective binding of poly-U. Ribosomes from seven rats fed basal diet bound ^{3}H-poly-U to an extent of 3550 ± 398 dis/min/150 μg of ribosomes, whereas ribosomes from seven rats fed 3′-Me-DAB bound ^{3}H-poly-U to an extent of 6626 ± 646 dis/min/150 μg of ribosomes. Greater binding of poly-U by 3′-Me-DAB ribosomes probably reflected a somewhat larger monosome population [8].

As shown in Table 2, other hepatocarcinogens, when fed to rats through 4 weeks, exerted effects upon free hepatic ribosomal populations that were similar to those exerted by 3′-Me-DAB feeding, i.e. they, too, interfered with poly-U stimulation of phenylalanine incorporation. This phenomenon was not uniquely associated with hepatocarcinogen feeding. For instance, feeding 4′-methyl-DAB, a weak hepatocarcinogen [67], did not result in the production of defective ribosomes. On the other hand, α-NIT, a compound known to induce hyperplasia of bile ducts [68, 69] but not hepatocarcinogenic [69], readily produced defective ribosomes. Thus, it was clear that interference with poly-U-stimulation of phenylalanine incorporation was induced by other hepatocarcinogens, but was not a phenomenon restricted to hepatocarcinogens.

The phenomenon of inhibited poly-U-directed phenylalanine incorporation after hepatocarcinogen feeding was not restricted to female Holtzman rats. Feeding the five hepatocarcinogens listed in Table 2 to male Fischer rats also resulted in marked inhibition of poly-U-directed phenylalanine incorporation by free ribosomes. On the other hand, free ribosomes isolated from female Fischer rats and male Holtzman rats seemed more resistant, because 4 weeks of hepatocarcinogen feeding exerted no appreciable influence upon poly-U-directed phenylalanine incorporation. After 8 weeks, however, inhibition was observed with three of the five hepatocarcinogens, i.e. 3′-Me-DAB, 4′-F-DAB and thioacetamide.

DISCUSSION

Data presented herein showed that a consistent consequence arising during the feeding of 3′-Me-DAB to rats was the development of a population of hepatic ribosomes which had limited ability to catalyze poly-U-directed phenylalanine incorporation. Data also were obtained which suggested that this consequence could be expected when other hepatocarcinogens were fed to other rat species. Clearly the ribosomal entity of the protein biosynthetic mechanism was affected by 3′-Me-DAB. This observation is not without precedence, for disturbance of the protein synthetic mechanism in target tissues during or subsequent to oncogenesis may ultimately become a common biochemical lesion of cancer [70]. More relevant to the present study, however, was the observation than DMN administration to rats inhibited the ability of hepatic ribosomes to catalyze poly-U-directed phenylalanine incorporation [32]. This observation was confirmed recently in a study with ribosomal subunits that were re-combined into 80S couples [45]. Inhibition of protein synthesis in ethionine-treated rats [71] also was associated principally with a ribosomal alteration [65, 71].

The emergence of this particular ribosomal population during the feeding of hepatocarcinogens to rats, irrespective of sex or species, lends credence to a conclusion that the change was correlated with the animal's risk for cancer. Three considerations temper such a conclusion: (a) α-NIT, a non-carcinogen [69], caused a similar ribosomal change (see Table 2); (b) the ribosomal change was induced with greater difficulty in the liver of rats that were probably more susceptible to hepatocarcinogenesis (although male Holtzman rats are more susceptible to liver cancer induced with 3′-Me-DAB [67] or 2-AAF [72], 8 weeks of feeding was necessary for the development of ribosomal changes); and (c), Wool *et al.* [73] have shown that diabetes inhibits the ability of muscle ribosomes to catalyze poly-U-directed phenylalanine incorporation into protein (it should be noted, however, that this inhibition was dependent upon both the concentration of poly-U and magnesium). Thus, it could be concluded that the phenomenon that emerges from these data was unrelated to the onset of malignant neoplasia. On the other hand, mechanisms of translations now are well enough understood that inhibited poly-U-directed phenylalanine incorporation can be attributed to disturbances involving initiation [74, 75], elongation [76, 77] or termination [78]. In view of this, perhaps additional study will better correlate specific translational disturbances and the animal's risk for cancer.

REFERENCES

1. E. C. Miller and J. A. Miller, *Pharmac. Rev.* **18,** 805 (1966).
2. J. A. Miller and E. C. Miller, *Lab. Invest.* **15,** 217 (1966).
3. E. Farber, *Cancer Res.* **28,** 1859 (1968).
4. C. Heidelberger, *Eur. J. Cancer* **6,** 161 (1970).
5. J. A. Miller, *Cancer Res.* **30,** 559 (1970).
6. C. Heidelberger, *Fedn Proc.* **32,** 2154 (1973).
7. K. R. Porter and C. Bruni, *Cancer Res.* **19,** 997 (1959).
8. B. Ketterer, S. J. Holt and P. Ross-Mansell, *Biochem. J.* **103,** 692 (1967).
9. D. Svoboda and J. Higginson, *Cancer Res.* **28,** 1703 (1968).
10. B. Flaks, *Chem. Biol. Interact.* **2,** 129 (1970).
11. W. H. Butler, *Chem. Biol. Interact.* **4,** 49 (1972).

12. D. J. Williams, R. P. Clark and B. R. Rabin, *Br. J. Cancer* **27,** 283 (1973).
13. K. R. Porter, *J. Histochem. Cytochem.* **2,** 346 (1954).
14. G. E. Palade, *J. biophys. biochem. Cytol.* **1,** 59 (1955).
15. H. Bloemendal, W. S. Bont and E. L. Benedetti, *Biochim. biophys. Acta* **87,** 177 (1964).
16. D. S. R. Sarma, I. M. Reid, E. Verney and H. Sidransky, *Lab. Invest.* **27,** 39 (1972).
17. P. N. Campbell, O. Greengard and B. A. Kernot, *Biochem. J.* **74,** 107 (1960).
18. T. Peters, Jr., *J. biol. Chem.* **237,** 1186 (1962).
19. J. R. Sargent and P. N. Campbell, *Biochem. J.* **96,** 134 (1965).
20. C. M. Redman, P. Siekevitz and G. E. Palade, *J. biol. Chem.* **241,** 1150 (1966).
21. T. Hallinan, C. N. Murty and J. H. Grant, *Life Sci.* **7,** Part 2, 225 (1968).
22. M. Takagi and K. Ogata, *Biochem. biophys. Res. Commun.* **33,** 55 (1968).
23. C. M. Redman, *J. biol. Chem.* **244,** 4308 (1969).
24. M. C. Ganoza and C. A. Williams, *Proc. natn. Acad. Sci. U.S.A.* **63,** 1370 (1969).
25. S. J. Hicks, J. W. Drysdale and H. N. Munro, *Science, N.Y.* **164,** 584 (1969).
26. M. Takagi, T. Tanaka and K. Ogata, *Biochem. biophys. Acta* **217,** 148 (1970).
27. H. Glaumann, *Biochim. biophys. Acta* **224,** 206 (1970).
28. M. Takagi and K. Ogata, *Biochem. biophys. Res. Commun.* **42,** 125 (1971).
29. D. G. Puro and G. W. Richter, *Proc. Soc. exp. Biol. Med.* **138,** 399 (1971).
30. T. Tanaka and K. Ogata, *J. Biochem. Tokyo* **70,** 693 (1971).
31. E. Farber, in *Advances in Cancer Research* (Eds. A. Haddow and S. Weinhouse) Vol. 7, p. 389. Academic Press, New York (1963).
32. T. Hultin and E. Arrhenius, in *Advances in Enzyme Regulation* (Eds. G. Weber and C. E. F. Weber) Vol. 3, p. 389. Pergamon Press, New York (1965).
33. H. V. Gelboin and R. Bates, *Publ. Hlth Rep., Wash.* **81,** 782 (1966).
34. H. V. Gelboin, in *Advances in Cancer Research* (Eds. A. Haddow and S. Weinhouse) Vol. 10, p. 1. Academic Press, New York (1967).
35. A. O. Hawtrey and L. D. Nourse, *Biochem. J.* **98,** 682 (1966).
36. J. G. Meyer-Bertenrath, *Chem. abstr.* **67,** 72363b (1967).
37. S. Villa-Trevino and D. D. Leaver, *Biochem. J.* **109,** 87 (1968).
38. M. Staehelin, *Biochim. biophys. Acta* **174,** 713 (1969).
39. D. W. John and L. L. Miller, *Biochem. Pharmac.* **18,** 1135 (1969).
40. T. Hultin, *Biochem. Pharmac.* **20,** 1009 (1971).
41. P. B. Lundeen, G. S. Banks and R. W. Ruddon, *Biochem. Pharmac.* **20,** 2522 (1971).
42. E. S. Reynolds, *Biochem. Pharmac.* **21,** 2555 (1972).
43. A. Sarasin and Y. Moule, *Fedn Eur. Biochem. Soc. Lett.* **29,** 329 (1973).
44. L. N. Vernie, W. S. Bont and P. Emmelot, *Cancer Res.* **31,** 2189 (1971).
45. A. Delpino and U. Ferrini, *Eur. J. Cancer* **9,** 245 (1973).
46. G. Medes, B. Friedman and S. Weinhouse, *Cancer Res.* **16,** 57 (1956).
47. T. Hallinan and H. N. Munro, *Q. Jl exp. Physiol.* **50,** 93 (1965).
48. G. Blobel and V. R. Potter, *J. molec. Biol.* **26,** 279 (1967).
49. J. W. Littlefield and E. B. Keller, *J. biol. Chem.* **224,** 13 (1957).
50. K. Tsukada, T. Moriyama, O. Doi and I. Lieberman, *J. biol. Chem.* **243,** 1152 (1968).
51. E. B. Keller and P. C. Zamecnik, *J. biol. Chem.* **221,** 45 (1956).
52. K. Moldave, in *Methods in Enzymology* (Eds. L. Grossman and K. Moldave) Vol. 12, Part B, p. 721. Academic Press, New York (1968).
53. T. W. Conway and F. Lipmann, *Proc. natn. Acad. Sci. U.S.A.* **52,** 1462 (1964).
54. K. Moldave and L. Skogerson, in *Methods in Enzymology* (Eds. L. Grossman and K. Moldave) Vol. 12, Part A, p. 478. Academic Press, New York (1967).
55. A. T. Jagendorf and M. Avron, *J. biol. Chem.* **231,** 277 (1958).
56. E. H. Allen and R. S. Schweet, *J. biol. Chem.* **237,** 760 (1962).
57. R. L. Miller and R. Schweet, *Archs Biochem. Biophys.* **125,** 632 (1968).
58. R. W. Wannemacher, Jr., W. L. Banks, Jr. and W. H. Wunner, *Analyt. Biochem.* **11,** 320 (1965).
59. G. A. Bruno and J. E. Christian, *Analyt. Chem.* **33,** 1216 (1961).
60. E. T. Bush, *Analyt. Chem.* **35,** 1024 (1963).
61. E. Volkin and W. E. Cohn, in *Methods in Biochemical Analysis* (Ed. D. Glick) Vol. 1, p. 287. Interscience, New York (1954).
62. P. B. Moore, *J. molec. Biol.* **18,** 8 (1966).
63. J. E. Dahlberg and R. Haselkorn, *J. molec. Biol.* **24,** 83 (1967).
64. I. M. Reid, D. S. R. Sarma and H. Sidransky, *Lab. Invest.* **25,** 141 (1971).
65. R. Kisilevsky, H. Shinozuka, E. L. Benedetti, K. H. Shull and E. Farber, *Lab. Invest.* **28,** 8 (1973).
66. G. M. Williams and T. Hultin, *Cancer Res.* **33,** 1796 (1973).
67. J. A. Miller and E. C. Miller, in *Advances in Cancer Research* (Eds. J. P. Greenstein and A. Haddow) Vol. 1, p. 339. Academic Press, New York (1953).
68. M. Lopez and L. Mazzanti, *J. Path. Bact.* **69,** 243 (1955).
69. M. R. McLean and K. R. Rees, *J. Path. Bact.* **76,** 175 (1958).
70. S. Weinhouse, *Fedn. Proc.* **32,** 2162 (1973).
71. R. Kisilevsky, *Biochim. biophys. Acta* **272,** 463 (1972).
72. E. K. Weisburger and J. H. Weisburger, in *Advances in Cancer Research* (Eds. J. P. Greenstein and A. Haddow) Vol. 5, p. 331. Academic Press, New York (1958).
73. I. G. Wool, W. S. Stirewalt, K. Kurihara, R. B. Low, P. Bailey and D. Oyer, in *Recent Progress in Hormone Research* (Ed. E. B. Astwood) Vol. 24, p. 139. Academic Press, New York (1968).
74. D. P. Leader, I. G. Wool and J. J. Castles, *Proc. natn. Acad. Sci. U.S.A.* **67,** 523 (1970).
75. D. P. Leader and I. G. Wool, *Biochim. biophys. Acta* **262,** 360 (1972).
76. F. Ibuki and K. Moldave, *J. biol. Chem.* **243,** 44 (1968).
77. P. Rao and K. Moldave, *J. molec. Biol.* **46,** 447 (1969).
78. A. K. Falvey and T. Staehelin, *J. molec. Biol.* **53,** 21 (1970).