functioning maximally in both groups. Therefore the total amount of ethanol metabolized during the first 10–12 hr should have been greater in group 1 than in group 3 if MEOS were functioning. The fact that the two blood ethanol curves were virtually superimposable from 10 hr onward suggests that, in fact, MEOS was not contributing to the ethanol disappearance.

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REFERENCES

1. S. Mallov and J. L. Bloch, *Am. J. Physiol.* **184,** 29 (1956).
2. R. D. Hawkins and H. Kalant, *Pharmac. Rev.* **24,** 67 (1972).
3. C. S. Lieber, *N. Engl. J. Med.* **288,** 356 (1973).
4. H. Theorell, *Experientia* **21,** 553 (1965).
5. L. Goldberg and U. Rydberg, *Biochem. Pharmac.* **18,** 1749 (1969).
6. D. Lester and G. D. Benson, *Science, N.Y.* **169,** 282 (1970).
7. J. C. Morgan and N. R. DiLuzio, *Proc. Soc. exp. Biol. Med.* **134,** 462 (1970).
8. G. O. Bustos, H. Kalant, J. M. Khanna and J. Loth, *Science, N.Y.* **168,** 1598 (1970).
9. R. Blomstrand and L. Forsell, *Life Sci.* **10,** part II, 523 (1971).
10. O. Johnson, O. Hernell, G. Fex and T. Olivecrona, *Life Sci.* **10,** part II, 553 (1971).
11. R. Domanski, D. Rifenberick, F. Stearns, R. M. Scorpio and S. A. Narrod, *Proc. Soc. exp. Biol. Med.* **138,** 18 (1971).
12. R. Nordmann, C. Ribiere, H. Rouach and J. Nordmann, *Revue Étud. clin. biol.* **17,** 592 (1972).
13. J. M. Khanna, H. Kalant, J. Loth and F. Seymour, *Biochem. Pharmac.* **23,** 3037 (1974).
14. W. H. Butler, H. M. Maling, M. G. Horning and B. B. Brodie, *J. Lipid Res.* **2,** 95 (1961).
15. E. Van Handel, *Clin. Chem.* **1,** 249 (1961).
16. A. E. LeBlanc, *Can. J. Physiol. Pharmac.* **46,** 665 (1968).
17. M. G. Horning, E. A. Williams, H. M. Maling, B. B. Brodie and W. M. Butler, Jr., *Biochem. biophys. Res. Commun.* **3,** 635 (1960).
18. A. B. Makar and G. J. Mannering, *Biochem. Pharmac.* **19,** 2017 (1970).
19. H. M. Maling, B. Highman, J. M. Hunter and W. M. Butler, in *Biochemical Factors in Alcoholism* (Ed. R. P. Maickel), p. 185. Pergamon Press, Oxford (1967).
20. P. E. Stokes, in *The Biology of Alcoholism* (Eds. B. Kissin and H. Begleiter), vol. 1, p. 397. Plenum, New York (1971).
21. J. S. Jenkins and J. Connally, *Br. med. J.* **2,** 804 (1968).
22. S. Bellet, L. Roman, O. de Castro and M. Herrera, *Metabolism* **19,** 664 (1970).
23. H. Kalant, R. D. Hawkins and C. Czaja, *Am. J. Physiol.* **204,** 849 (1963).
24. C. S. Lieber and L. M. DeCarli, *J. biol. Chem.* **245,** 2505 (1970).

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Stabilization of total and free ribosomes associated with 3-methylcholanthrene-induced adult rat liver growth

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Several investigators [1–4] have suggested that the accumulation and distribution of ribosomes within the cytoplasm play an important role in the modulation of protein synthesis which accompanies most situations of accelerated growth and development. While it is well established that increases in ribosomal RNA synthesis are responsible for much of the ribosome formation observed during rapid growth, there are few reports on the role of ribosomal RNA degradation in this process. Similarly, there is little information on the degradation rates of ribosomes in their two topographic states in the cell, i.e. free in the cytoplasm or bound to the endoplasmic reticulum, during induced growth. A complete understanding of the mechanism(s) of ribosome accumulation and distribution during growth and development requires that such studies be undertaken.

In rats, liver growth and an accumulation of cellular RNA follow the administration of certain drugs such as the barbiturate, phenobarbital and the polycyclic hydrocarbon, 3-methylcholanthrene (3-MC) [5–7]. Although RNA synthesis is stimulated shortly after adult rats are injected with 3-MC [7, 8], these increases in RNA synthesis are insufficient, in themselves, to account for the accumulation of RNA which accompanies the subsequent liver growth [7]. We [7] and others [9] have proposed that RNA stabilization may also be associated with 3-MC-induced adult rat liver growth. In the present experiments, we have investigated this contention more directly by studying the effect of 3-MC administration on the turnover of total and free rat liver ribosomes. Our results indicate that the half-life of ribosomes from 3-MC-treated rats is increased when the

Table 1. Liver weights of adult male rats injected with 20 mg/kg body weight of 3-methylcholanthrene (3-MC) or corn oil (CO)

Days after injection	Liver wt (g)		
	CO	3-MC	3-MC/CO
1	6·29 ± 0·34*	7·74 ± 0·36	1·23†
2	6·98 ± 0·38	8·07 ± 0·41	1·16
3	6·88 ± 0·30	8·21 ± 0·36	1·19†
4	7·10 ± 0·15	8·25 ± 0·14	1·16†
5	7·66 ± 0·31	8·02 ± 0·39	1·05
8	8·48 ± 0·18	8·52 ± 0·13	1·00

* Average ± standard error of the mean. N = four rats.
† P value = 0·05 or less.

liver hypertrophy is most pronounced and, further, that the half-life of both total and free ribosomes is prolonged equally by the drug.

Forty-eight male Charles River rats weighing about 200 g were given an injection of 4 μCi [^{14}C]orotic acid (Schwarz/Mann; sp. act., 42 μCi/mole), followed 2 hr later by an injection of 20 μmoles [^{12}C]orotic acid. Two hr after receiving the unlabeled orotic acid, the rats were injected with either 3-MC in corn oil (20 mg/kg of body weight) or the vehicle alone. All injections were given intraperitoneally. At 24-hr intervals after 3-MC or corn oil administration, the fasted animals were sacrificed by decapitation and their livers were rapidly excised, trimmed and weighed. All subsequent operations were performed at 0–3°.

Liver homogenates of 12·5% (w/v) were prepared in TKM buffer (50 mM Tris–HCl, pH 7·5, 25 mM KCl and 5 mM $MgCl_2$) containing 0·25 M sucrose, 2 mg/ml of yeast tRNA and 20 μg/ml of polyvinylsulfate. The homogenate was filtered through cheesecloth and approximately equal portions of the filtrate were used to prepare total and free ribosomes according to the methods of Venkatesan and Steele [10] and of Blöbel and Potter [11] respectively. The post-nuclear and post-mitochondrial supernatants were layered on top of two-step discontinuous gradients consisting of 2 ml of 0·5 M sucrose over 3 ml of 2·0 M sucrose (both in TKM buffer) and then centrifuged at 107,000 g for 22 hr in a Beckmann 40 or 50 Ti rotor. The ribosomal pellets were analyzed immediately or after storage at −20° for up to 1 week.

Ribosomal pellets were resuspended by gentle hand homogenization in 2 ml of 5 mM Tris–HCl, pH 7·5, and the resultant suspension was centrifuged at 5000 g (max) for 10 min. After measuring the absorbance of the supernatant fraction at 260 nm, aliquots were treated with a final concentration of 10% trichloroacetic acid (TCA), filtered through glass fiber discs (Whatman, GF/C), washed with 5% TCA, dried and counted in a toluene scintillation fluor which contained 0·5% 2,5-diphenyloxozole and 0·03% 1,4-bis-2(4-methyl-5-phenyloxazolyl)-benzene in a Beckman scintillation counter with an efficiency of about 67 per cent.

The concentration of ribosomes was calculated from an extinction coefficient of $E_{1\,cm}^{1\%}$ of 125 at 260 nm [12]. DNA and RNA were extracted from homogenates, subcellular fractions and ribosomes by the Schmidt–Thannhauser procedure and were measured by the diphenylamine and orcinol reaction, respectively, with deoxyadenosine monophosphate and yeast rRNA as the appropriate standards.

Liver weights were compared by means of the large-sample Student's t-test of Fisher. Regression lines and half-lives of ribosomes were computed from a least squares fit of the data.

Contamination of the post-nuclear supernatant fraction with even small amounts of highly labeled nuclei or nuclear RNA can appreciably alter the specific activity of the total ribosomes isolated therefrom [13]. Virtually 100 per cent of the cellular DNA sedimented with the nuclei after low speed centrifugation of the Triton X-100-treated homogenate. The reddish pellet also contained 9 per cent of the cellular RNA, or about twice the amount found in highly purified nuclei [11, 14], suggesting co-sedimentation of small amounts of cellular debris or ribosomes. No appreciable leakage of nuc-

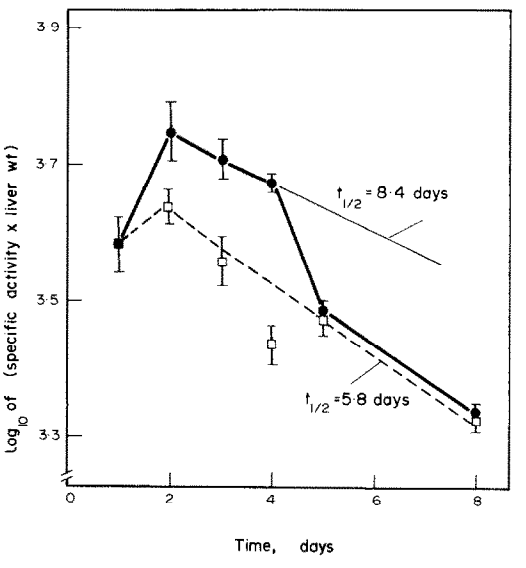

Fig. 1. Effect of 3-MC administration on the turnover of rat liver ribosomes. The figure shows the product, to the $\log_{10}$, of the specific activity of liver ribosomes (dis./min per mg ribosomes) and the liver weight (g) as a function of time (days) after corn oil (□---□) or 3-MC (●—●) administration. The data are expressed as the average ± standard error of the mean. Four animals were used for each point.

lear ribonucleoprotein particles occurred during the isolation of nuclei, since the ribosomes derived from post-nuclear supernatants of rats injected 20 min prior to sacrifice with [^{14}C]orotic acid contained no radioactivity. Approximately 76 per cent of the cellular RNA was recovered in the total ribosomal pellets isolated from the deoxycholate-treated post-nuclear supernatant, a value which compares favorably with that obtained by others using the same [10] or different [11, 14] techniques.

Total and free ribosomes had similar A_{260}/A_{280} ratios of about 1·75, suggesting highly purified preparations [15]. In experiments not shown, sedimentation profiles of both total and free polysomes revealed fairly large monomer and dimer peaks, at least partly a reflection of the relatively long centrifugation period [16], as well as aggregates up to nanosomes. However, the total polysome profile also contained a greater proportion of more slowly sedimenting aggregates. While ribonuclease activity in the post-nuclear supernatant, even in the presence of the two ribonuclease inhibitors, probably contributed in large part to this observation, other workers [17, 18] have reported similar differences in the sedimentation pattern of bound (in our total ribosome fraction) and free ribosomes isolated from post-mitochondrial supernatants.

Accurate half-life values of ribosomes can only be obtained when the labeling period is short relative to the interval of the measurements and when reutilization of the isotope is negligible. In these experiments the injection of [^{14}C]orotic acid was followed 2 hr later with an injection of a 200-fold greater amount of [^{12}C]orotic acid. In agreement with other workers using a similar regimen [19, 20], radioactivity in the nucleotide pools of control and experimental rat livers was negligible by 28 hr after [^{14}C]orotic acid injection and was completely absent thereafter (data not shown).

The decline in the specific activity of ribosomes must also take into account the dilution of labeled ribosomes by unlabeled species which occurs during normal and induced liver growth during the course of the experiment. Table 1 shows that during the experimental period, control liver increased by 35 per cent in weight. The livers of 3-MC-treated rats were clearly larger than those of controls, especially during the first 4 days after drug administration. By 5–8 days, the liver weights of corn oil- and 3-MC-treated rats were the same. Since the content of total ribosomal RNA, which accounted for 76 per cent of the cellular RNA, increased in proportion to liver weight in both control and experimental rats (data not shown), we, as well as others [20–23], have corrected for the liver growth by multiplying the specific activity of the ribosomes by the liver weight.

Since the specific activity of total and free ribosomes from control or experimental rat liver was the same at each time interval, we averaged these values. Corrected for the increases in liver weight, the specific activity of total and free ribosomes isolated from adult rat liver at various times after corn oil or 3-MC administration is shown in Fig. 1. The specific activity of liver ribosomes reached a maximum by 2 days after corn oil or 3-MC injection, and then declined thereafter. In corn oil-treated controls, the specific activity of liver ribosomes conformed to a single exponential decay pattern with a half-life of 5·8 days. This value is well within the range reported by most other workers [13, 20, 22–26]. In 3-MC-pretreated rats, the decay pattern was more complex, but could best be fitted with two straight lines, one having a slope which corresponded to a half-life of 8·4 days, the other to a half-life of about 5·8 days. Thus, the half-life of hepatic ribosomes from 3-MC-pretreated rats was increased when drug-induced liver hypertrophy was most marked, and was virtually identical when experimental and control liver weights were the same. This ribosomal RNA stabilization in the liver of 3-MC-pretreated rats may have come about as a result of the diminished hepatic ribosomal RNase levels [9].

These experiments demonstrate that ribosomal RNA stabilization contributes, at least in part, to the accumulation of cellular RNA associated with 3-MC-induced adult rat liver growth. In this system then, alterations in the rates of ribosome synthesis, as well as of ribosome degradation, vary to regulate the ribosome content of the liver; thus both processes help maintain the steadystate concentrations of these entities within the cell. Since the majority of total cellular hepatic ribosomes are membrane-bound [11, 14, 17], these experiments also indicate, in agreement with others [13, 22, 23, 26], that the half-life of bound and free liver ribosomes in control rats is the same. The administration of 3-MC then, appears to prolong the half-life of both ribosomal populations to an equal degree. This suggests, but in no sense proves (see Ref. 1), that liver ribosomes, once formed (see Ref. 27), normally exist in a dynamic equilibrium between their two physical states in the cell, and that this equilibrium is not detectably altered even when the ribosomes content of the liver changes.

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REFERENCES

1. J. R. Tata, in *Karolinska Symposia on Research Methods in Reproductive Endocrinology* (Ed. E. Diczfalusy), Vol. 6, pp. 192–224, Karolinska Institutet, Stockholm (1973).
2. H. L. Cooper, in *Biochemistry of Cell Division* (Ed. R. Baserga), pp. 91–112. Charles C Thomas, Springfield, Ill. (1969).
3. C. P. Stanners and H. Becker, *J. Cell Physiol.* **77**, 31 (1971).
4. A. M. Novi and R. Baserga, *Lab. Invest.* **26**, 540 (1972).
5. T. S. Argyris, *J. Pharmac. exp. Ther.* **164**, 405 (1968).
6. T. S. Argyris, in *Repair and Regneration* (Eds. J. E. Dunphy and W. VanWinkle), pp. 201–216. McGraw-Hill, New York (1969).
7. J. Hopkinson and T. S. Argyris, *Lab. Invest.* **29**, 342 (1973).
8. R. C. Wilson, J. S. Wortham and H. V. Gelboin, *Adv. Enzyme Regulat.* **5**, 385 (1967).
9. C. R. Pousada and M. C. Lechner, *Biochem. Pharmac.* **21**, 2563 (1972).
10. N. Venkatesan and W. J. Steele, *Biochem. biophys. Acta* **277**, 646 (1972).
11. G. Blöbel and V. R. Potter, *J. molec. Biol.* **26**, 279 (1967).

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12. M. H. Schreier and T. Staehelin, *J. molec Biol.* **73,** 329 (1973).
13. G. Blöbel and V. R. Potter, *Biochim. biophys. Acta* **166,** 48 (1968).
14. M. Zweig and J. W. Grisham, *Biochim. biophys. Acta* **246,** 70 (1971).
15. H. Noll, in *Techniques in Protein Biosynthesis* (Eds. P. N. Campbell and J. R. Sargeant), Vol. 2, pp. 101–207. Academic Press, New York (1969).
16. T. E. Webb, G. Blöbel and V. R. Potter, *Cancer Res.* **24,** 1229 (1964).
17. N. Vankatesan and W. J. Steele, *Biochem. biophys. Acta* **287,** 526 (1972).
18. G. Blöbel and V. R. Potter, *J. molec. Biol.* **26,** 293 (1967).
19. A. A. Hadjiolov, *Biochim. biophys. Acta* **119,** 547 (1966).
20. J. N. Loeb, R. R. Howell and G. M. Tomkins, *Science, N.Y.* **149,** 1093 (1965).
21. J. Seifert, J. Vacha and H. Remmer, *Proc. Eur. Soc. Drug Toxic.* **12,** 253 (1971).
22. M. Izawa and S. Ichii, *J. Biochem.* **74,** 623 (1973).
23. H. Nordgren and U. Stenram, *Hoppe-Seyler's Z. physiol. Chem.* **353,** 1832 (1972).
24. C. A. Hirsch and H. H. Hiatt, *J. biol. Chem.* **241,** 5936 (1966).
25. R. V. Quincy and S. H. Wilson, *Proc. natnl. Acad. Sci. U.S.A.* **64,** 981 (1969).
26. R. K. Mishra, J. F. Wheldrake and L. A. W. Feltham, *Biochim. biophys. Acta* **281,** 393 (1972).
27. C. N. Murty and H. Sidransky, *Biochim. biophys. Acta* **335,** 226 (1974).

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Effect of parathyroid hormone and adenosine 3′,5′-monophosphate on renal carbonic anhydrase*

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Parathyroid hormone (PTH) increases the renal excretion of phosphate [1]. In addition, PTH causes a modest increase (up to about 7 per cent of filtered load) of bicarbonate excretion [2]. It has been postulated that the effect of PTH and cyclic AMP, which also mimics the action of PTH on phosphate excretion, produces excretion of bicarbonate by direct inhibition of renal carbonic anhydrase [3]. PTH (10 units/ml) and cyclic AMP (0·4 mM) have been reported to inhibit rat renal carbonic anhydrase, after 20 min pre-incubation of the homogenate at 37°, up to 33 and 60 per cent respectively [3]. It has been further reported that 0·4 mM cyclic AMP but not PTH produced 85 per cent inhibition of carbonic anhydrase purified from rat renal cortex after 2-min pre-incubation [4]. However, Puschett and Goldberg [2] reported lack of effect of PTH on erythrocyte carbonic anhydrase. Beck and Goldberg [5] did not find any inhibition of erythrocyte carbonic anhydrase with 150 units/ml of PTH or 3 mM cyclic AMP. Therefore, it was thought to be of interest to reinvestigate the effect of PTH and cyclic AMP on renal carbonic anhydrase.

Table 1. Effect of parathyroid hormone on carbonic anhydrase in buffered supernatant fraction of rat renal cortex*

Parathyroid hormone (units/ml)	Carbonic anhydrase† (c.u./ml)	pH
0	1·89 ± 0·10	7·32
10	1·85 ± 0·12	5·92

* Samples were incubated for 20 min at 37° and the pH was recorded after cooling the samples.

† Values given here are mean ± standard deviation of three experiments.

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Rats of the Wistar strain were anesthetized with ether, and their kidneys were perfused free of blood with 0·9% NaCl solution through renal arteries. The medulla and cortex were separated, weighed and homogenized in 10 vol. of 50 mM Tris buffer, pH 7·4, or distilled water with a glass homogenizer. The homogenates were centrifuged at 3000 rev/min for 30 min in a Sorvall (model RC-2B) centrifuge.

The supernatant fraction was mixed with PTH or acetic acid or a freshly prepared solution of cyclic AMP. The samples were incubated at 37° in a water bath with a shaker for 20 min. After incubation, the samples were cooled and their pH was recorded. The carbonic anhydrase activity of the samples was then determined by the method of Maren *et al.* [6]. The variability of the method was 10 per cent.

PTH used was Parathyroid Injection of Eli Lilly & Co. Cyclic AMP was obtained from Sigma Chemical Co.

Table 1 shows that incubation of renal cortical carbonic anhydrase with 10 units/ml of parathyroid hormone had no effect on the enzyme activity, when the enzyme was in a buffer and the pH did not drop below 5. It may be pointed out that the pH of the Parathyroid Injection (containing 100 units/ml) was 3·3. In the case of enzyme prepared in distilled water, its pH dropped to 4·6 after adding 10 units/ml of parathyroid hormone (Table 2). In this case, the incubation of the reaction mixture for 20 min at 37° produced a 33 per cent decrease in carbonic anhydrase activity. The sample without pre-incubation did not show any change in enzyme activity. The results of Table 2 suggest that acidic