two opposing factors are acting, with ethanol not only decreasing synthesis of all proteins through its influence on liver, but simultaneously and perhaps non-specifically, stimulating fibrinogen synthesis (which is generally considered to be easily enhanced). Although the latter view might seem to be supported by the observation that fibrinogen synthesis is increased by treatment with Aminosol (and especially when there is no increase in synthesis of albumin) it is not supported by the fact that ethanol has no significant effect on the synthesis of fibrinogen in the presence of Aminosol. Nor is there support for this view by our results for batch II (Table 3) in which, as previously remarked, a very substantial decrease in albumin synthesis is accompanied by normal fibrinogen synthesis that is not significantly different from that in batch III, in which albumin synthesis was only moderately decreased.

When the pattern of change in these syntheses is compared with the alterations in plasma protein concentrations seen in experimental and clinical protein malnutrition there appears to be a similarity, in that malnutrition also causes a decrease of albumin and transferrin concentrations (Platt *et al.*, 1964; Peetom, 1962). Preliminary studies show that short-term protein deprivation in the rat has relatively little effect on fibrinogen synthesis (K. N. Jeejeebhoy, A. Bruce-Robertson, J. Ho & U. Sodtke, unpublished work).

In summary, our results suggest that ethanol inhibits plasma protein synthesis in a selective way, and that this change, and the ultrastructural ones seen at higher dosage, can be prevented by giving amino acids. The exact mechanism by which ethanol operates needs elucidation.

We continue to be grateful to Dr. A. S. McFarlane for ever helpful advice and discussion concerning the method, and to Dr. Harold Kalant for much useful information, advice and discussion concerning the ethanol aspect. In this respect we thank also Dr. Yedy Israel and Dr. David Bloxam. A. B.-R. thanks Schering Corp. Ltd., Pointe Claire, Que., Canada, for its Travel Award, which enabled him to visit Dr. McFarlane. This work was generously supported by the Alcohol and Drug Addiction Research Foundation of Ontario, and the control work by the Medical Research Council (Canada) Grants MA.3204 and MA.785 and by a capital grant from the Bickell Foundation.

References

Bruni, C. & Porter, K. R. (1965) *Amer. J. Pathol.* **46**, 691
Gordon, A. H. & Louis, L. N. (1963) *Biochem. J.* **88**, 409
Herdson, P. B., Garvin, P. J. & Jennings, R. B. (1964) *Amer. J. Pathol.* **45**, 157
Jeejeebhoy, K. N., Samuel, A. M., Singh, B., Nadkarni, G. D., Desai, H. G., Borkar, A. V. & Mani, L. S. (1969) *Gastroenterology* **56**, 252
Jeejeebhoy, K. N., Bruce-Robertson, A., Sodtke, U. & Foley, M. (1970) *Biochem. J.* **119**, 243
Koj, A. & McFarlane, A. S. (1968) *Biochem. J.* **108**, 137
Lieber, C. S. & Rubin, E. (1969) *N. Engl. J. Med.* **280**, 705
McFarlane, A. S. (1963*a*) *Biochem. J.* **89**, 277
McFarlane, A. S. (1963*b*) *J. Clin. Invest.* **42**, 346
McFarlane, A. S. (1964) *Mammalian Protein Metab.* **1**, 297
McFarlane, A. S., Irons, L., Koj, A. & Regoeczi, E. (1965) *Biochem. J.* **95**, 536
McKinley, J. E., Gilbert, D. B., Chao, P.-Y. & Reeve, E. B. (1970) *Amer. J. Physiol.* **218**, 491
Mollenhauer, H. H. (1964) *Stain Technol.* **39**, 111
Munro, H. N. (1970) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **29**, 1490
Pease, D. C. (1964) *Histological Techniques for Electron Microscopy*, p. 216, Academic Press Inc., New York
Peetoom, F. (1962) *Trop. Geogr. Med.* **14**, 193
Platt, B. S., Heard, C. R. C. & Stewart, R. J. C. (1964) *Mammalian Protein Metab.* **2**, 445
Porta, E. A., Koch, O. R. & Hartroft, W. S. (1970) *Exp. Mol. Pathol.* **12**, 104
Rubin, E. & Lieber, C. S. (1967) *Gastroenterology* **52**, 1
Trump, B. F., Smuckler, E. A. & Benditt, E. P. (1961) *J. Ultrastruct. Res.* **5**, 343

APPENDIX

Rates of Plasma Protein Synthesis by Deconvolution

By K. H. NORWICH
Department of Physiology and Institute of Bio-Medical Electronics and Engineering, University of Toronto, Toronto 181, *Ont., Canada*

(*Received* 31 *August* 1971)

The plasma specific radioactivity of arginine guanidine ^{14}C-labelled protein has been measured at various times after the injection of labelled protein precursor ($Na_2{}^{14}CO_3$). The resulting 'appearance' curve is a balance between the rate of synthesis and delivery of new protein and the rate of destruction and distribution of new plus existing protein.

The measured appearance curve can be corrected to give a measure solely of the rate of synthesis of the plasma protein concerned, if the amplitude of the curve at every point is increased by an amount that compensates for the destruction and distribution processes. The decrease of plasma specific radioactivity of labelled plasma protein after an injection of exogenously labelled protein is termed the 'disappearance' curve, and the method of correcting the appearance curve by using the disappearance curve is shown to take the form of a deconvolution.

The problem presenting itself was to correct the measured appearance curves of specific radioactivity of arginine guanidine ^{14}C of the plasma protein concerned for concomitant ^{14}C-protein loss and dispersion, to yield an undistorted arginine guanidine ^{14}C appearance curve in the plasma. As frequently occurs, the removal of a distorting influence from a series of measurements can take the form of a deconvolution. Since the molecular kinetics involved only tracers, and the traced substances were in steady state, the assumption of a linear system is probably warranted (Norwich & Hetenyi, 1971). The linear system concerned may be defined operationally as follows. The input is defined as the rate of appearance of arginine guanidine ^{14}C-labelled albumin, fibrinogen or transferrin in the plasma [d.p.m.·time^{-1}], and the output is defined as the plasma specific radioactivity of the corresponding substance [d.p.m.·mass^{-1}·time^{-1}]. Thus the 'system' acts upon the incoming labelled material to disperse and degrade it. The problem can then be translated into the following simple terms: given the output of the system, compute the input.

Let $X(t)$ be a general input to a linear system, and $Y(t)$ the corresponding output. Let $H(t)$ be the output resulting from a unit impulse input; that is, $H(t)$ is the unit impulse response function of the system. If the Laplace transform is designated by the operator $\mathscr{L}$ then from the theory of linear systems (e.g. Cheng, 1959):

$$\mathscr{L}X(t)=\frac{\mathscr{L}Y(t)}{\mathscr{L}H(t)} \tag{1}$$

$Y(t)$ represents the measured curve of plasma specific radioactivity of arginine guanidine ^{14}C. In order to compute $X(t)$, the undistorted form, one must first obtain $H(t)$, which is accomplished by means of a concurrent experiment: namely the measurement of the disappearance of ^{125}I-labelled protein from the plasma after an impulse (Dirac pulse) input of mass M^* [d.p.m.] at zero time.

The experimental points of the plasma ^{125}I-labelled protein specific radioactivity disappearance curve were fitted to a linear combination of two exponential functions with real, negative indices, by means of a digital computer, employing the least squares criterion, by a method similar to that of Hazelrig *et al.* (1963). $H(t)$, the response of the system/d.p.m. of injected tracer, was thus obtained in the form:

$$H(t)=\frac{1}{M^*}(C_1'\mathrm{e}^{-a_1t}+C_2'\mathrm{e}^{-a_2t}) \tag{2}$$

It was thought more desirable to express the input function, $X(t)$, in the same units as the output function, which necessitated dividing $X(t)$ by the mass, M, of the 'pool' into which the newly-synthesized arginine guanidine ^{14}C protein is extruded. That is:

$$M=\frac{M^*}{C_1'+C_2'} \tag{3}$$

$$\text{Define } F(t)\ [\text{d.p.m.}\cdot\text{mass}^{-1}\cdot\text{time}^{-1}]\equiv\frac{X(t)}{M} \tag{4}$$

$$C_1\equiv\frac{C_1'}{C_1'+C_2'} \tag{5a}$$

$$C_2\equiv\frac{C_2'}{C_1'+C_2'} \tag{5b}$$

Hence the following equations are readily derived:

$$C_1+C_2=1 \tag{5c}$$

$$C_2-C_2^2=C_1-C_1^2=C_1C_2 \tag{5d}$$

By substituting eqns. (2), (3), (4), (5*a*) and (5*b*) into eqn. (1), one obtains:

$$\mathscr{L}F(t)=\frac{\mathscr{L}Y(t)}{\mathscr{L}(C_1\mathrm{e}^{-a_1t}+C_2\mathrm{e}^{-a_2t})} \tag{6}$$

To obtain $Y(t)$, the experimental points of the arginine guanidine ^{14}C appearance curve were fitted to a linear combination of three exponential functions with real, negative indices by a method similar to that for double exponential fitting. All computations were performed on IBM 7094 II or 360 computers. The function obtained was of the form:

$$Y(t)=\sum_{i=1}^{3}A_i\mathrm{e}^{-b_it} \tag{7}$$

and from the constraints of the experiment:

$$\sum_{i=1}^{3}A_i\doteqdot 0 \tag{8}$$

The remainder of the techniques involved in this deconvolution process are quite similar to those used by Wraight (1969).

Substituting eqn. (7) into eqn. (6):

$$\mathscr{L}F(t)=\frac{\mathscr{L}\sum_{i=1}^{3}A_i\mathrm{e}^{-b_it}}{\mathscr{L}(C_1\mathrm{e}^{-a_1t}+C_2\mathrm{e}^{-a_2t})} \tag{9}$$

The Laplace transforms on the right-hand side of eqn. (9) may now be evaluated explicitly [see Abramowitz & Stegun (1964) or any standard set of tables.]:

$$\mathscr{L}F(t)=\frac{\sum_{i=1}^{3}\frac{A_i}{s+b_i}}{\frac{C_1}{s+a_1}+\frac{C_2}{s+a_2}} \qquad (10)$$

For simplicity, define

$$\beta\equiv C_1 a_2+C_2 a_1 \qquad (11)$$

Substituting eqns. (5*c*) and (11) into eqn. (10) and simplifying:

$$F(t)=\sum_{i=1}^{3}A_i\left[1+\frac{a_1-b_i}{s+b_i}+\frac{a_2-\beta}{s+\beta}+\frac{(a_1-b_i)(a_2-\beta)}{(s+b_i)(s+\beta)}\right] \qquad (12)$$

Upon taking inverse transforms of both sides of eqn. (12) and applying eqn. (8), one obtains:

$$F(t)=\sum_{i=1}^{3}A_i\left[\left\{(a_1-b_i)-\frac{(a_1-b_i)(a_2-\beta)}{b_i-\beta}\right\}e^{-b_i t}+\left\{(a_2-\beta)+\frac{(a_1-b_i)(a_2-\beta)}{b_i-\beta}\right\}e^{-\beta t}\right] \qquad (13)$$

Defining two more constants:

$$\alpha\equiv\beta-a_1-a_2 \qquad (14)$$

$$\gamma\equiv\frac{C_1 C_2(a_1-a_2)^2}{\beta} \qquad (15)$$

and substituting eqn. (5*d*) into eqn. (13), one obtains:

$$F(t)=\sum_{i=1}^{3}A_i\left[\left\{-b_i-\alpha+\frac{\beta\gamma}{b_i-\beta}\right\}e^{-b_i t}-\frac{\beta\gamma}{b_i-\beta}e^{-\beta t}\right] \qquad (16)$$

Thus eqn. (16) gives the function $F(t)$ explicitly in terms of parameters that are known from the experimental results. $F(t)$, then, defines the corrected appearance function of arginine guanidine ^{14}C-labelled albumin, fibrinogen or transferrin. The cumulative appearance of tracer [d.p.m.·mass^{-1}] can readily be computed from $\int_0^t F(\tau)\,d\tau$.

The constants α, β and γ may be regarded as defined solely for computational convenience, or they may be given physical significance by regarding the system as composed of three compartments (Matthews, 1957). Within the system of compartments defined by her it may be seen that:

$$\alpha=-K_{12}-K_{13}$$
$$\beta=K_{31}$$
$$\gamma=K_{13}$$

where the K's are rate constants [Matthews (1957): eqns. (19), (20) and (21)].

I thank Dr. S. Zelin for his careful proof-reading of this appendix. This work has been supported by a generous grant from the Medical Research Council (Canada).

References

Abramowitz, M. & Stegun, I. A. (1964) *Handbook of Mathematical Functions*, p. 1022, U.S. Government Printing Office, Washington D.C.

Cheng, D. K. (1959) *Analysis of Linear Systems*, p. 232, Addison–Wesley, Reading, Pa.

Hazelrig, J. B., Ackerman, E. & Rosevear, J. W. (1963) *Proc. Annu. Conf. Eng. Med. Biol. 16th.* **5**, 8

Matthews, C. M. E. (1957) *Phys. Med. Biol.* **2**, 36

Norwich, K. H. & Hetenyi, G., Jr. (1971) *Biophysik* **7**, 169

Wraight, E. P. (1969) *Phys. Med. Biol.* **14**, 403