Response of tyrosine-α-ketoglutarate transaminase activity to ribonucleic acids

The induction of certain liver cytoplasmic enzymes, such as tyrosine-α-ketoglutarate transaminase[1] has been inferred to occur by virtue of increased net synthesis of the apoenzyme. This conclusion was drawn from a demonstration of induction of TAKT *in vivo* in the relative absence of coenzyme[2]. Additional indirect information emphasizes the importance of exogenous protein for the maintenance of TAKT activity[3]. To the present time, stimulation of TAKT activity following injection of L-tyrosine and/or hydrocortisone[1] has not been related directly to RNA, a relationship which would be expected to follow from investigations into the mechanism of protein synthesis in animal tissues. However, increased ^{32}P incorporation into RNA has been observed to follow induction of tryptophan peroxidase[4], and increased incorporation of labelled valine into partially purified fractions containing tryptophan peroxidase has been reported following induction of this enzyme[5].

In this laboratory it was considered that total liver RNA present at the peak of induction (5 h after injection of inducer) might contain a high-molecular-weight fraction which could, by itself, accomplish TAKT induction. It was found, instead, that total rat liver RNA prepared by known methods[6,7], which was soluble in water, accomplished the induction *in vivo*. Furthermore, induction was accomplished in a similar fashion following intraperitoneal injection of highly purified, highly polymerized RNA fractions from yeast, following injection of several commercial RNA preparations known to contain chiefly low-molecular-weight components, and finally, following injection of synthetic mixtures of 2′,3′-nucleotides simulating the ratios found in RNA. Peak stimulation of TAKT activity was observed 4 h after injection of RNA in each case (Table I). The optimal concentration of RNA 4 h after injection is signified by a sigmoid dependence curve which levels off at 20 mg/100 g body weight. These experiments were controlled by injections of equal volumes of saline, or by injection of an equal mass of heparin, or by the use of untreated animals. Slight stimulation was obtained from saline or heparin control reactions compared to the untreated controls. Heparin produced a higher response than saline, yet it was not considered to be comparable to RNA or nucleotide mixtures. The stimulation of TAKT activity by RNA appears to be mediated by the adrenal gland (or hydrocortisone), but this dependence is not absolute when nucleotide mixtures replace RNA in adrenalectomized animals. Stimulation is often found in the latter case, although it is not equivalent to the level found when the animal is intact or when replacement with hydrocortisone is instituted. Moreover, commercial sources of DNA have been poorly active in stimulating TAKT activity in whole animals. These findings emphasize the importance of studying the differential rates of breakdown of RNA and DNA in the peritoneal fluid. Studies with creatine phosphate and ATP± glucose indicate that RNA does not cause the stimulation of TAKT activity by providing energy sources in the form of nucleotide derivatives.

In adrenalectomized animals the greatest stimulation of TAKT activity 4 h after injection is observable when hydrocortisone, RNA or the nucleotide mixture, and essential amino acids are supplied simultaneously through the intraperitoneal route.

Abbreviations: TAKT, tyrosine-α-ketoglutarate transaminase; ATP, adenosine triphosphate; RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

TABLE I

EFFECTS OF YEAST RNA PREPARATIONS ON RAT-LIVER TAKT ACTIVITY AS A FUNCTION OF TIME AFTER INTRAPERITONEAL INJECTION

30 mg/100 g body wt. purified high-molecular-weight yeast RNA.

Time after injection (h)	*Number of animals*	*Average body weight (g)*	*ANN* × 10⁸*	*Average TAKT activity***			
				per g liver	*per 100 g body weight*	*per organ weight*	*per ANN × 10⁻⁸*
0.5							
Untreated	3	370	1.4	13.9	3.8	226	9.9
Heparin	3	344	1.9	16.4	4.8	283	8.6
RNA	3	366	2.1	14.8	4.0	247	7.0
2.0							
Untreated	3	312	1.4	13.7	4.4	169	10.1
Heparin	3	292	1.8	16.9	5.8	207	9.7
RNA	3	292	1.9	45.7	15.6	537	24.0
4.0							
Untreated	4	256	1.7	7.2	2.8	82	4.2
Heparin	4	288	2.7	11.4	3.9	124	4.2
RNA	4	278	2.2	101.0	36.2	1120	46.0
5.0							
Untreated	4	313	1.8	13.5	4.2	205	7.6
Heparin	8	280	2.1	22.7	7.8	313	10.5
RNA	12	289	2.5	86.5	31.1	1160	37.8
17.0							
Untreated	3	244	2.3	18.2	7.4	170	8.1
Heparin	3	256	2.3	10.7	4.2	129	4.8
RNA	3	260	2.0	16.2	6.2	181	8.1

* ANN = average number of liver nuclei/g determined by the method of BASS *et al*[8].

** Change in absorbancy at 310 mμ/10 min in a standard assay system[1] at 25° using 0.3 ml of a supernatant preparation. Total volume, 3.5 ml.

Part of this work was performed at Rutgers University, New Brunswick, New Jersey, with the technical assistance of VALENTINA WOJCIECHOWSKI. The high-molecular-weight yeast RNA fractions were prepared by Dr. F. F. DAVIS, Rutgers University, using a salt fractionation procedure which will be published. This investigation was supported in part by research grant A-4187 from The National Institute of Arthritis and Metabolic Diseases, N.I.H., U.S.P.H.S.

Biochemical Laboratory, Division of Cardiology, GERALD LITWACK
Philadelphia General Hospital and The Graduate School of Medicine,
University of Pennsylvania, Philadelphia 4, Pa. (U.S.A.)

1 E. C. C. LIN AND W. E. KNOX, *Biochim. Biophys. Acta*, 26 (1957) 85.
2 E. C. C. LIN, M. CIVEN AND W. E. KNOX, *J. Biol. Chem.*, 233 (1958) 1183.
3 G. LITWACK, P. S. EBERT, S. YANKELOWITZ AND M. W. TAYLOR, *Enzymologia*, 20 (1959) 183.
4 P. FEIGELSON, M. FEIGELSON AND C. FANCHER, *Biochim. Biophys. Acta*, 32 (1959) 133.
5 P. GROS, J. COURSAGET AND G. P. TALWAR, *Bull. soc. chim. Belges*, 65 (1956) 107.
6 K. S. KIRBY, *Biochem. J.*, 64 (1956) 405.
7 A. GIERER AND G. SCHRAMM, *Nature*, 177 (1956) 702.
8 A. D. BASS, A. H. MCARDLE AND J. W. GRISHAM, *Science*, 128 (1956) 372.

Received May 28th, 1960