acetyl moiety of amplexoside A and comparison of the paper chromatographic characteristics[4] of this derivative to an authentic sample. Final proof for the cinnamate moiety, on the other hand, came about when cinnamic acid was isolated from the hydrolysis reaction of the acylgenin. The isolated cinnamic acid, λ_{max} (0.05 *N* KOH) 267 nm; λ_{max} (0.1 *N* H_2SO_4) 270 nm; ν_{max} 1698^{-1}, was confirmed by comparison to a known sample by paper chromatography[8].

The genin portion was established as sarcostin since the isolated material had m.p. 145–150/260–263° (reported[6] m.p. 145–150/257–263°), typical colour reactions with hydrochloric acid[9] and 84% sulfuric acid[10], and the same Rf as a known sample when compared in two TLC systems[11,12] and one paper chromatography system[3].

The sugar portion was placed at C-3 by analogy with other molecules of this type[11]. It was found to consist of asclepobiose and digitoxose by comparison with known specimens in one TLC system[3] and three paper chromatography systems[3,13].

Résumé. A partir de la racine d'*Asclepias amplexicaulis* on a isolé un nouveau glycoside de la série prégnane, avec une potentialité d'action anti-cancer. Il possède l'aglycone: 12-cinnamoyl-20-*O*-acétylsarcostine. Les sucres asclépobiose et digitoxose se trouvent á C-3.

A. M. Ahsan, D. M. Piatak[14] and P. D. Sorensen

Department of Chemistry and Department of Biological Sciences, Northern Illinois University DeKalb (Illinois 60115, USA),
16 February 1973.

[8] E. C. Bate-Smith in *Partition Chromatography* Biochem. Soc. Symposia, No. 3, (Ed. R. T. Williams; University Press, Cambridge 1949), p. 62.
[9] J. W. Conforth and J. C. Earl, J. chem. Soc., *1939*, 737.
[10] E. Abisch, Ch. Tamm and T. Reichstein, Helv. chim. Acta *42*, 1014 (1959).
[11] K. Jäggi, H. Kaufmann, W. Stöcklin and T. Reichstein, Helv. chim. Acta *50*, 2457 (1967).
[12] S. Sakuma, H. Ishizone, R. Kasai, S. Kawanishi and S. Shoji, Chem. pharm. Bull. Tokyo *19*, 52 (1971).
[13] F. Kaiser, Chem. Ber. *88*, 556 (1955).
[14] We gratefully acknowledge samples of asclepobiose and sarcostin from Prof. T. Reichstein and his workers.

Activity of Cytidine Triphosphate Synthetase in Normal and Neoplastic Tissues

The pool of free cytidine nucleotides in the animal cell is small compared with those of the other ribonucleotides. Further, it was reported that in tumour cells the amount of these nucleotides is higher than in non-proliferating tissues[1] and that cytidine triphosphate (CTP) decreases to a very low level by the end of postnatal brain development[2]. It may be suggested that CTP, or some step in the CTP producing pathway, is a limiting factor in tissue growth and/or RNA synthesis[1,2].

To test this hypothesis we investigated the activity of CTP synthetase (EC 6.3.4.2, UTP: ammonia ligase (ADP)) in several normal and neoplastic tissues. The presence of this enzyme in animal tissues is proved in soluble extracts from Novikoff hepatoma[3] and liver[3–6].

Materials and methods. Liver, kidney, brain, heart, spleen, testis and blood were obtained from adult male rats. Transplantable myeloma MOPC-21 (grown for about 20 days) and Ehrlich ascites cells were obtained from mice, and rhabdomyosarcoma and skeletal muscle from hamsters. Tissues were removed immediately after decapitation of the animals, cut into pieces and rinsed with ice-cold 0.25 *M* sucrose containing 50 m*M* *Tris*-Cl (pH 7.6) and 1 m*M* EDTA. They were homogenized in the same solution with a glass-Teflon homogenizer and the homogenates (25–30%, w/v) were centrifuged in the cold for 80 min at 105,000 *g*. The supernatant fractions obtained were stored in 2 ml aliquots at −20 °C, and were used within 3 weeks after preparation. During this storage, the changes of enzyme activity were insignificant.

The enzyme activity was determined in a standard incubation mixture (see [3–6]) which contained in a final volume of 1.0 ml: 20 μmoles $MgCl_2$, 10 μmoles ATP, 0.4 μmole GTP, 5 μmoles L-glutamine, 10 μmoles phosphoenolpyruvate, 0.22 μmole [4-^{14}C] UTP (8.4×10^6 counts/min/μmole) and soluble cell fraction containing 2–12 mg protein. The components of the mixture were brought to pH 7.5–7.6 with 1 *M* *Tris*. The incubation was carried out at 37 °C and terminated at different time intervals up to 60 min, after which a slightly modified scheme of Hurlbert and Kammen[3,7] for nucleotide hydrolysis, separation and estimation was followed.

The radioactivity was measured in a Packard Tri-Carb Spectrometer with 2.0 ml aqueous samples and 10 ml of a dioxan-based scintillator fluid, counting efficiency being about 60%. Protein was determined according to Lowry et al.[8], using crystalline bovine serum albumin as a standard.

Activity of CTP synthetase in soluble cell fractions obtained from normal and neoplastic tissues[a]

Tissue	CTP synthetase (nmoles/h/mg protein)
Blood	0
Sceletal muscle	0.4
Liver	0.7
Heart	1.2
Kidney	1.5
Brain	1.6
Spleen	1.8
Rhabdomyosarcoma	4.0
Myeloma	5.1
Testis	12.2
Ehrlich ascites tumour cells[b]	16.0

[a] For the origin of the tissues and isolation of the soluble cell fractions see 'Materials and methods'. The Figures are mean values from 3 to 10 independent samples. [b] The cells were collected 9–12 days after implantation.

[1] P. Mandel, Bull. Soc. Chim. biol. *49*, 1491 (1967).
[2] S. Edel and G. Poirel, Bull. Soc. Chim. biol. *48*, 935 (1966).
[3] R. B. Hurlbert and H. O. Kammen, J. biol. Chem. *235*, 443 (1960).
[4] C. R. Savage and H. Weinfeld, J. biol. Chem. *245*, 2529 (1970).
[5] D. D. Genchev, C. r. Acad. bulg. Sci. *23*, 435 (1970).
[6] D. D. Genchev and A. A. Hadjiolov, FEBS Letters *3*, 147 (1969).
[7] H. O. Kammen and R. B. Hurlbert, Cancer Res. *19*, 654 (1959).
[8] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, J. biol. Chem. *193*, 265 (1951).

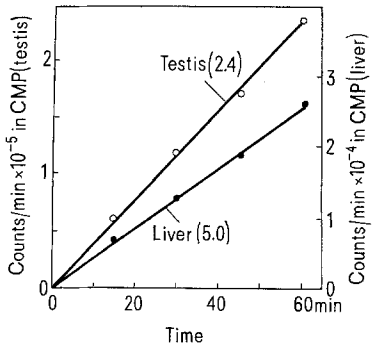

Fig. 1. Time course of the conversion of [^{14}C]UTP into CTP catalyzed by the soluble cell fractions from testis and liver. The numbers in brackets represent the amount of protein present in the standard incubation mixtures (see 'Materials and methods'). CTP was isolated as CMP[3,7].

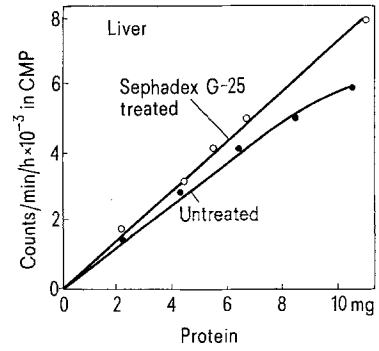

Fig. 2. Dependence of the conversion of [^{14}C]UTP into CTP on the amount of soluble cell fraction protein present in the medium. Untreated rat liver supernatant was obtained as described under 'Materials and methods'. Immediately before the incubation an aliquot of this fraction was passed through Sephadex G-25 column equilibrated with 0.02 *M Tris*-Cl (pH 7.6) and eluted with the same buffer. The results are expressed as counts/min found in the CMP fraction after 1 h incubation.

Results and discussion. Previous investigations[3,6] have shown that CTP synthetase cannot be measured in whole tissue homogenates. Therefore the soluble cell fraction was used in this study. Under the employed conditions of enzyme assay, the amount of radioactivity from [^{14}C] UTP converted into CTP did not usually exceed 10% of total in each sample. The conversion proceeded linearly with time (Figure 1) at a rate directly depending on the amount of soluble cell fraction protein present (Figure 2). However, when larger quantities of this fraction were used, a slight deviation from linear dependence was observed. This was due to the introduction into the system of endogenous low-molecular weight compounds, as revealed by control experiments in which soluble cell extracts were purified by gel filtration on Sephadex G-25 (Figure 2).

The results of the determination of CTP synthetase are shown in the Table. As the extent of cellular disruption of the different tissues may be uneven, the results are expressed as nmoles UTP aminated to CTP/h/mg protein of the soluble cell fraction, rather than per unit wet tissue weight. However, if calculations are made according to the latter basis, approximately the same ratios among the tissue activities will hold (cf. also [9]). It can be seen from the Table that the activities of various tissues, as determined under optimal conditions in vitro, differ considerably (by a factor of more than 30). The highest rate of CTP synthesis was found in soluble fractions from ascites cells and testis. Rhabdomyosarcoma and myeloma also showed high activity, while moderate or low activity was displayed by spleen, brain, kidney, heart, liver and muscle. Blood extract was essentially devoid of activity. It has to be noted that the actual values for the CTP synthetase activity of the neoplastic tissues studied are probably higher than those reported in the Table, since the percent of the dead cells or necrotic regions could not be quantitatively assessed and consequently no corrections for their presence were made.

Although the results given in the Table were obtained using crude extracts throughout, the significant differences observed in the CTP synthetase activity of examined tissues are not due to the presence of endogenous low-molecular weight substances. Analogous results were obtained with the soluble fractions purified by gel filtration on Sephadey G-25. These differences cannot be explained by the interference of side reactions since 1. omission of phosphoenolpyruvate from the standard medium decreased the incorporation of radioactivity into the CMP fraction by no more than 20–30%, and 2. as shown previously[3,5,6] in this system, the radioactivity recovered as free bases and nucleosides was negligible.

The above results clearly show that CTP synthetase is an ubiquitous enzyme and that rough correlation does exist between its activity and the growth rate or mitotic activity of the tissues. The relatively high capacity for CTP synthesis displayed by brain may reflect an intense RNA biosynthesis, rather than cell division in some parts of the central nervous system.

If the present results are compared on the same basis with those reported for the activity and tissue distribution of the enzymes catalyzing the preceding steps of pyrimidine biosynthesis[5,9–12], it will be noted that the activity of CTP synthetase is extremely low. It is comparable only to that of the first enzyme of the pathway, viz. carbamoyl phosphate synthetase, which appears to be of regulatory importance in mammals[11,12]. Our results indicate that CTP synthetase may also be involved in cellular control mechanisms of tissue growth and/or RNA synthesis.

Résumé. L'activité de la synthétase CTP dans différents tissus normaux et cancéreux a été étudiée. Les résultats indiquent qu'elle est élevée dans les tissus en prolifération.

D. D. GENCHEV

Institute of Biochemistry, Bulgarian Academy of Sciences, Sofia 13 (Bulgaria), 15 February 1973.

[9] J. PAUSCH, D. KEPPLER and K. DECKER, Biochim. biophys. Acta 258, 395 (1972).
[10] J. E. YOUNG, M. D. PRAGER and I. C. ATKINS, Proc. Soc. exp. Biol. Med. *125*, 860 (1967).
[11] M. TATIBANA and K. ITO, J. biol. Chem. *244*, 5403 (1969).
[12] M. C. M. YIP and W. E. KNOX, J. biol. Chem. *245*, 2199 (1970).
[13] Acknowledgements. The author would like to express his gratitude to Professor A. A. HADJIOLOV for valuable discussions during this study.