

TABLE I. DPN-CONCENTRATIONS IN ASCITES TUMOR CELLS AND LIVER TISSUE OF MICE 10 HR AFTER INTRAMUSCULAR ADMINISTRATION OF NICOTINAMIDE AND NICOTINIC ACID (170 MG/KG)

	No. of animal	Ascites tumor cells (μg of DPN/ml of packed cells)	Liver tissue (μg of DPN/g of wet weight)
A. Control animals	1	208	193
	2	194	263
	3	194	232
	4	197	215
	Mean	198	225
	S.D.	± 7	± 30
B. Nicotinamide	5	317	1451
	6	—	1351
	7	285	1260
	8	290	1520
	Mean	297 ($\pm 50\%$)	1395 ($\pm 521\%$)
	S.D.	± 17	± 114
C. Nicotinic acid	9	227	430
	10	230	530
	11	—	550
	12	235	655
	Mean	230 ($\pm 16\%$)	541 ($\pm 141\%$)
	S.D.	± 4	± 92

Two samples were lost by overalkalization. *t*-Test comparisons are significant at or beyond the 0.01 level of probability for B against A, C against A, and B against C, with respect to both ascites tumor cells and liver tissue. Figures in parentheses represent average increases over the control values.

REFERENCES

1. J. PREISS and P. HANDLER, *J. Biol. Chem.* **233**, 488 (1958).
2. T. A. LANGAN, N. O. KAPLAN and L. SHUSTER, *J. Biol. Chem.* **234**, 2161 (1959).
3. J. M. ROITT, *Biochem. J.* **63**, 300 (1956).
4. H. HOLZER und H. KRÖGER, *Klin. Wschr.* **36**, 677 (1958).
5. H. HOLZER, D. BUSCH und H. KRÖGER, *Hoppe-Seylers Zeit. physiol. Chem.* **313**, 184 (1958).
6. H. HOLZER und H. J. BOLTZE, *Zeit. für Krebsforsch.* **64**, 64 (1961).

Separation of thymidine and its mono-, di- and tri-phosphate by paper chromatography

(Received by Editors 6 June 1961; Received by Publishers 3 July 1961)

THYMIDINE (TDR) and its various 5'-phosphorylated derivatives (thymidylic acid (TMP), thymidine diphosphate (TDP), and thymidine triphosphate (TTP)) have been separated readily from each other by column chromatography;^{1, 2} however, it has been difficult to obtain clean separations using paper chromatography.³ Although TDR and TMP were easily separable on paper, TDP and TTP, under the same conditions, appreciably overlapped, and the results could be interpreted only in a semi-quantitative manner.³ Since paper chromatography often permits the separation of micro-quantities of materials, and is very convenient when dealing with a large number of samples, such a method for the efficient separation of TDR, TMP, TDP and TTP was sought. The present report describes a method, using Whatman cellulose paper AE30, which effectively separates the four compounds.

The paper strip to be used in the separation was washed successively with formic acid (0.1 N), ammonium formate (0.5 N) and water for 24 hr. After the addition, at the top of the dried paper strip, of the solution containing the thymine-derivatives, and re-drying, the first solvent, formic acid (0.01 N), was used to separate TDR from the other compounds. In this solvent system, TDR moved with the solvent front, which was permitted to travel almost to the end of the paper. The paper was re-dried before elution with the second solvent system, ammonium formate (0.06 N, pH 5.0), which was allowed to flow for about two-thirds of the distance traversed by the first solvent system. Such a

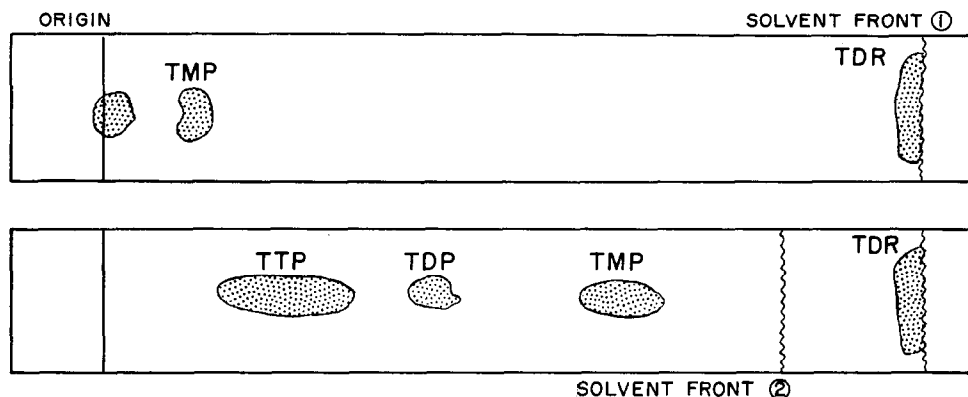


FIG. 1 Paper chromatograms

separation is desirable particularly when studying the metabolism of radioactive thymidine. The various phosphorylated derivatives of TDR, shown in the Fig 1, were located with the aid of an ultraviolet lamp; each component could be eluted with dilute hydrochloric acid (0.1 N) with essentially 100 per cent recovery. The minimum quantity of each compound which could be discerned clearly was 0.2 μ mole, although smaller quantities were detectable.

Acknowledgement—We are grateful to the Damon Runyon Memorial Fund for Cancer Research for the personal support of one of us (I.W.D.), while on leave from The Department of Medicine of The University of Edinburgh.

Department of Pharmacology,
Yale University School of Medicine,
New Haven, Connecticut, U.S.A.

I. W. DELAMORE
W. H. PRUSOFF

REFERENCES

1. R. B. HURLBERT, H. SCHMITZ, A. F. BROUW and V. R. POTTER, *J. Biol. Chem.* **209**, 23 (1954).
2. S. M. WEISSMAN, R. M. S. SMELLIE and J. PAUL, *Biochem et Biophys. Acta* **45**, 101 (1960).
3. F. J. BOLLUM and V. R. POTTER, *Cancer Res.* **19**, 561 (1959).

Inhibition of crotonyl coenzyme A-reducing activity of liver microsomes by 1:1:3-tricyano-2-amino-1-propene (U-9189)

(Received by Editors 20 July 1961; Received by Publishers 24 July 1961)

THE decreased capacity of diabetic rat liver to synthesize fatty acids has been related, in part, to the decreased activity of crotonyl coenzyme A (crotyl-CoA)-reducing enzymes in particle fractions from the liver.¹ We have found that the activity of this enzymic reaction in rabbit and rat liver microsomes