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

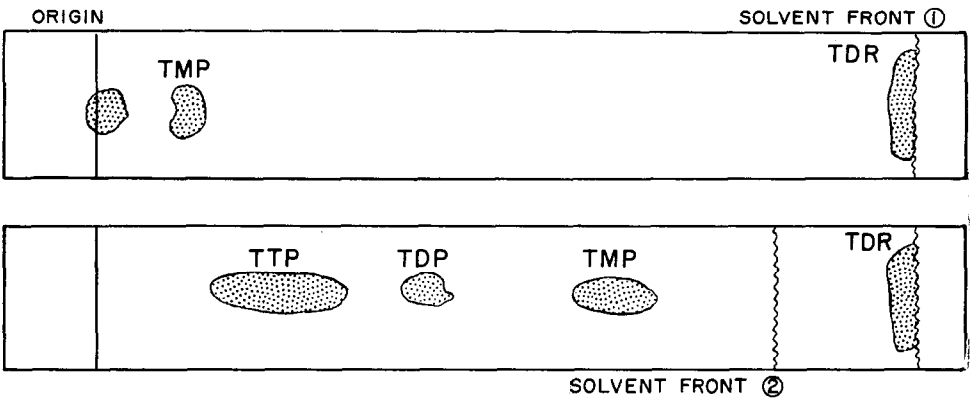


FIG. 1 Paper chromatograms

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 μmole, 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

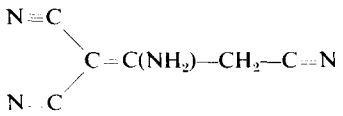
1. R. B. HURLBERT, H. SCHMITZ, A. F. BROUMM and V. R. POTTER, *J. Biol. Chem.* **209**, 23 (1954).
2. S. M. WEISSMAN, R. M. S. SMELLIE and J. PAUL, *Biochem et Biophys. Acta* **45**, 101 (1960).
3. F. J. BOLLUM and V. R. POTTER, *Cancer Res.* **19**, 561 (1959).

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 (crot-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

is inhibited by 1:1:3-tricyano-2-amino-1-propene (U-9189),[2] a compound also shown[3] to be an inhibitor of oxidative phosphorylation.



(U-9189)

The standard assay mixture consisted of 600 μmoles of Tris buffer, pH 7·5, 60 μmoles of $MgCl_2$, 0·20–0·25 μmoles of reduced triphosphopyridine nucleotide* (TPNH), a suspension of washed liver microsomes (collected in 0·25 M sucrose) in 0·1 M phosphate buffer, pH 7·5, with 0·5% sodium cholate, and water to 1·8 ml. After measuring any endogenous TPNH utilization, 0·2 ml (0·05 μmoles) of crot-CoA solution (prepared according to Goldman[4]) was added, and the decrease in absorbance at 340 mμ (compared to a mixture containing all ingredients except TPNH) was measured at 30-sec intervals for 5–10 min in the Beckman DU-spectrophotometer. All reagents were held at 37 °, and the cuvette compartment also was controlled at this temperature.

A rabbit liver microsomal preparation which utilized TPNH at a net of 1·3 mμmoles per min per mg protein was completely inhibited when U-9189 was present at 5×10^{-4} M. The inhibitions were noted when U-9189 was added either before substrate addition or after a 5-min measurement of activity in the complete system. At 5×10^{-5} M, U-9189 inhibited the system by about 10–15 per cent. If sufficient microsal suspension was present to produce a signifiant rate of TPNH utilization before substrate addition, U-9189, 5×10^{-4} M was without effect on this endogenous consumption.

Table 1 presents data from two preparations of rat liver microsomes in the presence of varying concentrations of U-9189 added prior to substrate. In another experiment, a concentration of $4·5 \times$

TABLE 1. EFFECT OF U-9189 ON CROTONYL CoA-REDUCING ACTIVITY IN RAT LIVER MICROSOMES

Final molar conc. U-9189	Experiment 1		Experiment 2	
	mμmoles TPNH utilized per min per mg protein	Per cent inhibition	mμmoles TPNH utilized per min per mg protein	Per cent inhibition
0	2·1		2·4	
5×10^{-4}	0·25	90		
4×10^{-4}			0	100
$2·5 \times 10^{-4}$	1·0	52	0·75	70
1×10^{-4}	1·4	35	1·8	25
$7·5 \times 10^{-5}$	1·5	30		
5×10^{-5}	1·6	25		

The assay system is described in the text. For a particular microsomal suspension, maximum variation of duplicate measurements of the utilization of TPNH was ±5 per cent.

10^{-4} M, following a 5-min measurement of activity in the complete system, resulted in complete cessation of TPNH utilization, while a concentration of 1×10^{-5} M reduced the rate by about 10–15 per cent.

Final concentrations of 10^{-3} M KCN, acetonitrile or malononitrile had no inhibitory effect, nor did two compounds† related to U-9189, $C_2H_5—O—\underset{\underset{O}{\|}}{C}—\underset{\underset{CN}{|}}{C}=\underset{\underset{NH_2}{|}}{C}—CH_3$ (U-14, 508), 4×10^{-4}M final concentration, and $C_2H_5—O—\underset{\underset{O}{\|}}{C}—\underset{\underset{CN}{|}}{C}=\underset{\underset{NH_2}{|}}{C}—\underset{\underset{O}{\|}}{C}—O—C_2H_5$ (U-13, 758), 9×10^{-4} M.

* Sigma Chemical Co. Solutions were prepared just before use and concentrations determined spectrophotometrically.

† Prepared by Dr. G. A. Youngdale.

In contrast to the effect on the utilization of TPNH in crot-CoA-reduction, U-9189 had little or on effect on glutathione reductase,* another system in which the reduced nucleotide is required. Concentrations between 5×10^{-4} M and 2×10^{-3} M exhibited variable and inconsistent inhibitions of from 10 to 25 per cent. The order of introduction of U-9189 was of no significance.

Thus, 1:1:3-tricyano-2-amino-1-propene not only is an inhibitor of an enzymic step important in the synthesis of fatty acids in liver, but also it may possess a specificity of activity which could facilitate elucidation of the mechanistic details of the reactions involved. Black and Hudson[5] have presented evidence for a flavin prosthetic group in yeast glutathione reductase, and Lynen recently reported[6] that flavin mononucleotide is an intermediate in the reduction by TPNH of α, β-unsaturated acyl CoA-compounds during fatty acid synthesis by yeast. Wakil has suggested[7] that precedence favours the flavin intermediate in TPNH-double bond reductions. If this generalization is valid, in the microsomal system it seems most probable that U-9189 is actually interfering with hydrogen-transfer from a flavin to the model substrate.

* A preparation from yeast obtained from Sigma Chemical Co.

Research Laboratories,
The Upjohn Company,
Kalamazoo, Michigan.

PAUL W. O'CONNELL
FLOYD S. EBERTS, JR.

REFERENCES

1. K. J. MATTHES, S. ABRAHAM and I. L. CHAIKOFF, *Biochim. Biophys. Acta* **37**, 181 (1960).
2. F. S. EBERTS, JR., *Arch. Biochem. Biophys.* In press.
3. F. S. EBERTS, JR., *Biochem. Pharmacol.* In press.
4. D. GOLDMAN, *J. Biol. Chem.* **208**, 345 (1956).
5. S. BLACK and B. HUDSON, *Biochem. Biophys. Res. Commun.*, **5**, 135 (1961).
6. F. LYNEN, *Intersociety Session on Lipid Metabolism, Federated Societies Meeting, Atlantic City* (1961).
7. S. WAKIL, *J. Lipid Res.*, **2**, 1 (1961).

SRS–S concentrates from normal swine lung without anaphylaxis

(*Received by Editors* 9 *August* 1961; *Received by Publishers* 29 *August* 1961)

THE release of a smooth muscle-stimulating substance, in addition to histamine, from the lung tissue of guinea pig during anaphylaxis has been reported,[1-5] and is of interest in research in allergy. This "substance" has not been proven by organic chemical data to be a single substance, but is characterized primarily by biological effects and the relation of biological response to chemical fractionation. This substance causes a prolonged contraction of isolated smooth muscle, in contrast to the rapid contraction caused by histamine; consequently, it has been designated "slow-reacting substance" or SRS[1, 2] or SRS-A[5] (A for anaphylaxis).

Previous methods[1-5] for extraction of SRS or SRS-A from guinea pigs required sensitization of the animals with antigen, usually egg albumin. The lungs were then removed, and anaphylaxis was induced by perfusion or incubation of the sensitized lungs with antigen solution. The aqueous effluents contained SRS and histamine only after anaphylaxis. The existence of SRS in these effluents was based on observations of the characteristic contraction of the guinea pig ileum after suppression of the action of histamine with antihistaminic agents.[2] In our study, this procedure served as the basis of an assay[5, 6] for activity of samples.

The preparation of concentrates of SRS for biological and chemical studies is greatly hampered by the small quantities available from the lungs of guinea pigs and by the sensitization requirement. Although the background research seemed to preclude the presence of SRS in normal lung tissue, lungs from swine have been investigated as a source for SRS, and very active concentrates have been prepared. In the absence of definitive characterizing data on SRS obtained from different sources and