viduals involved, no definite conclusions can be drawn from these observations and any explanation falls into the realm of speculation. It is suggestive, however, of a change in the infectivity of the virus in the process of dilution, perhaps concerned with a virus-antibody relationship.

Cortisone therapy appeared to have a definite and immediate beneficial effect in the three patients who were treated, but any conclusions concerning its value from this study would again be questionable since there was no adequate control group and the number of cases was small.

Summary. 1. Known icterogenic (SH) serum (J.W. strain), undiluted and diluted 10% in normal saline, was filtered through gradocol membranes of various average pore diameters down to 52 mμ, and tested in human volunteers. 2. Evidence has been presented that the strain of serum hepatitis virus used is small enough to pass through a 52 mμ A.P.D. membrane, thus indicating a size of 26 mμ or less. The limitations of the application of this method for determining the size of this virus are mentioned.

The author is indebted to Dr. John R. Paul for valuable advice and criticism.

1. Findlay, G. M., and MacCallum, F. O., *Proc. Roy. Soc. Med.*, 1938, v31, 799.

2. Beeson, P. B., Chesney, G., and McFarlan, A. M., *Lancet*, 1944, v1, 814.

3. Oliphant, J. W., *The Harvey Lecture Series*, 1943-1944, v39, 254.

4. Elford, W. J., *J. Path. and Bact.*, 1931, v34, 505.

5. Evans, A. S., PROC. SOC. EXP. BIOL. AND MED., 1951, v75, 809.

6. Elford, W. J., *Proc. Roy. Soc.* (London), 1933, vB112, 384.

7. Bugher, J. C., and Smithburn, K. C., *Bact. Proc.*, 1952, p83.

8. Koprowski, H., and Cox, H. R., *J. Immunol.*, 1947, v57, 239.

9. Unpublished experiments with Joseph L. Melnick.

Received August 25, 1952. P.S.E.B.M., 1952, v81.

In Vitro Conversion of Thiocyanate to Cyanide in the Presence of Erythrocytes.* (19810)

KERMIT L. PINES AND MARGARET M. CRYMBLE. (Introduced by R. F. Loeb.)
From the Department of Medicine, Columbia University College of Physicians and Surgeons, and Presbyterian Hospital, New York City.

The *in vivo* conversion of thiocyanate to cyanide has been reported recently by Goldstein and Rieders(1) in contrast to previous studies in which this reaction could not be demonstrated(2,3). Because thiocyanate has been widely used in the management of patients with hypertensive vascular disease, further study of this problem was undertaken in the course of investigation of thiocyanate-induced diuresis in man(4).

Methods. Cyanide was determined by the method of Gettler and Goldbaum(5). The material to be tested is acidified, heated to 90°C and aerated; the eluted hydrogen cyanide is carried to filter paper impregnated with ferrous sulfate, producing the Prussian blue reaction. The method is reported to be highly specific and sensitive to 0.0001 mg. For the purposes of the present study no effort was made to obtain sensitivity greater than 0.0005 mg. Standards were set up in whole blood and serum containing 0.0005, 0.001, 0.002, 0.003, 0.004, and 0.005 mg of cyanide;† the colored papers were preserved for comparison with the unknowns. The specificity of the method appeared to be adequate as judged by a limited number of observations. Repeated analyses of water, serum, plasma, and boiled whole blood to which varying amounts of thiocyanate had been added yielded no detectable cyanide. The development of the character-

* This study was supported in part by grants from the Albert and Mary Lasker Foundation and from the National Heart Institute (U.S.P.H.S.).

† The terms "cyanide" and "thiocyanate" refer to the anion only.

istic odor and the conversion of methemoglobin to cyanmethemoglobin (by spectrophotometric analysis) as well as the starch-iodine and copper-benzidine acetate tests(5a) confirmed the presence of cyanide in instances where the test was positive. Thiocyanate was determined by the method of Barker(6) adapted for use with the Coleman spectrophotometer. Methemoglobin was estimated by the method described by Evelyn and Malloy(7). Known amounts of potassium thiocyanate were introduced into test tubes containing 2 ml of heparinized whole blood or other fluid, or equivalent weights of tissue, at room temperature. The cyanide determination was completed in most instances within 10 minutes of the addition of thiocyanate. Measurements were also made on the fasting whole blood and serum of 2 hypertensive patients before and during administration of therapeutic doses of potassium thiocyanate. The analyses were repeated in one of these patients on another occasion at which time thiocyanate was given during the production of methemoglobinemia by oral doses of p-aminopropiophenone(8). Whole blood cyanide was estimated in 2 hypertensive subjects who had been given sodium nitroprusside, a depressor agent reported to give rise to thiocyanate in man(9).

Results. The introduction of potassium thiocyanate into heparinized human or rabbit whole blood *in vitro* was followed within 10 minutes in each 50 analyses by the appearance of measurable amounts of cyanide in the red blood cells. No detectable cyanide was observed in the plasma in these instances. The affinity of erythrocytes for this compound was further indicated by measurements on whole blood after the addition of cyanide; in these cases, cyanide first appeared in the plasma after the concentration in whole blood had been increased to 0.2 mg per 100 ml.

The conversion of thiocyanate to cyanide was also observed in hemolysed blood, but not in previously boiled whole blood. Cyanide was not detectable following the addition of thiocyanate to plasma, serum, water, rabbit liver homogenate or minced rabbit kidney. Nor were measurable amounts of cyanide obtained from solutions of thiocyanate in whole blood containing as little as 2.1% methemoglobin, presumably owing to the formation of stable cyanmethemoglobin (Table I).

TABLE I. Cyanide Concentrations Following the Introduction of Potassium Thiocyanate *In Vitro.*

Medium		Initial thiocyanate, mg/100 ml	Cyanide yield, mg/100 ml
Human whole blood		0	0
		5	.2
		20	.2
		100	.4
Human whole blood	boiled	50	0
	hemolysed	280*	.56*
	+ 2.1% methemoglobin	22	0
Human serum		20	0
		250	0
Water		20	0
		250	0
Rabbit	kidney, minced	110†	0
	liver, homogenized	125	0

* Corrected for dilution factor of 2.8.
† mg per 100 g.

TABLE II. Serial Serum Thiocyanate and Whole Blood Cyanide Levels in Hypertensive Patients Receiving Therapeutic Doses of Potassium Thiocyanate by Mouth.*

Patient	Day	Serum thiocyanate, mg/100 ml	Methemoglobin, %	Whole blood cyanide, mg/100 ml	Serum cyanide
1	1	0		0	0
	3	4		.05	0
	5	4.2		.05	0
2	1	.2		0	0
	3	3.6		.05	
	5	6.1		.1	
During induced methemoglobinemia					
2	1	.1	1.9	0	
	3	3.2	6.9	0	
	5	5.4	2.1	0	

* Determinations were made on fasting blood drawn just prior to the beginning of therapy, after two and after four days of thiocyanate administration.

Serial determinations of serum thiocyanate and whole blood cyanide during thiocyanate therapy (Table II) indicated that cyanide was present when thiocyanate concentrations were approximately half the recommended therapeutic level(6). As anticipated, no

cyanide was found in the blood of the patient with methemoglobinemia; it was observed, however, in the blood of both patients given nitroprusside coincident with the appearance in the serum of thiocyanate derived from the nitroprusside.

Discussion. These data confirm the observation of Goldstein and Rieders that thiocyanate is partly converted to cyanide, and indicate further that the reaction is rapid and occurs *in vitro* in the presence of a constituent of the red blood cell. The integrity of the erythrocyte is not essential to the conversion, and the system is heat labile. The evidence thus is compatible with the concept that the reaction is enzymatic, and that the responsible enzyme occurs in the red blood cell. The failure of the conversion to take place in the presence of rabbit liver or kidney is presumptive evidence that these tissues do not contain the enzyme. Lang has demonstrated the presence in liver homogenates of "rhodanase," an enzyme capable of irreversibly synthesizing thiocyanate from cyanide in the presence of colloidal sulfur or thiosulfate. No "rhodanase" was found in muscle or blood(10). It is conceivable, but unlikely in the absence of added sulfur, that the small quantities of cyanide formed from thiocyanate in liver preparations are rapidly reconverted by the "rhodanase" system, leaving none to be detected by the procedure used.

Although well below lethal concentrations, the levels of cyanide observed in the present study are sufficiently high to produce functional changes at least in the kidney. A dose of 0.03 mg of cyanide (0.05 mg of sodium cyanide) per 100 g of body weight has been reported to produce a sodium diuresis in dogs made resistant to mercurials(11). Estimates of the minimal lethal dose of cyanide range between 0.3 mg per 100 g of body weight in dogs(12) to 0.05 mg per 100 g in man(13). A blood level as low as 0.34 mg per 100 ml was observed by the latter authors in a fatal human case of cyanide poisoning. Blood levels in cases of non-fatal poisoning were not found in published reports, but it is probable that these would lie within the range of the concentrations observed following conversion from thiocyanate. Certainly the signs of toxicity from cyanide and thiocyanate are similar in animals and man, as previously noted(1).

The absence of detectable amounts of cyanide from the plasma of blood containing small amounts does not preclude the transfer of red cell cyanide to take part in reactions elsewhere. It is well known that cyanide combines with methemoglobin to form cyanmethemoglobin, a derivative with a characteristic absorption spectrum. This compound was formed within 10 to 15 minutes of the addition to methemoglobin of erythrocytes containing cyanide derived from thiocyanate.

Cyanide and thiocyanate have certain properties in common including their pattern of toxicity in animals and man(1), the production of sodium diuresis(4,11), and inhibition of carbonic anhydrase(14). These similarities support the suggestion(1) that cyanide may account in part for some effects, *in vitro* as well as *in vivo*, formerly ascribed to thiocyanate.

Summary. The conversion of thiocyanate to cyanide was confirmed, and evidence was presented indicating that this reaction occurs rapidly *in vitro* in the presence of a red blood cell constituent, possibly an enzyme. Physiologically significant concentrations of cyanide were observed in the blood of patients with serum thiocyanate values even below therapeutic levels. The evidence was felt to support the suggestion that the biological effects generally ascribed to thiocyanate may be caused in part by cyanide.

The authors gratefully acknowledge the advice and assistance of Dr. George A. Perera.

1. Goldstein, F., and Rieders, F., *Am. J. Physiol.*, 1951, v167, 47.

2. Goodman, L., and Gilman, A., *The Pharmacological Basis of Therapeutics*, The Macmillan Co., New York, 1941.

3. Sollman, T., *A Manual of Pharmacology*, W. B. Saunders, Phila., 1942.

4. Pines, K. L., and Perera, G. A., PROC. SOC. EXP. BIOL. AND MED., in press.

5. Gettler, A. O., and Goldbaum, L., *Analyt. Chem.*, 1947, v19, 270.

5a. Feigl, F., *Qualitative Analysis by Spot Tests*, Nordemann Publishing Co., New York, 1939.

6. Barker, M. H., *J.A.M.A.*, 1936, v106, 762.

7. Evelyn, K. A., and Malloy, H. T., *J. Biol. Chem.*, 1938, v126, 655.
8. Tepperman, J., Bodansky, O., and Jandorf, B. J., *Am. J. Physiol.*, 1946, v146, 702.
9. Page, I. H., *J.A.M.A.*, 1951, v147, 1311.
10. Lang, K., *Biochem. Z.*, 1933, v259, 243.
11. Farah, A., and Koda, F., *Fed. Proc.*, 1952, v11, 343.
12. Chen, K. K., and Rose, C. L., *J.A.M.A.*, 1952, v149, 113.
13. Gettler, A. O., and Baine, J. O., *Am. J. Med. Sci.*, 1938, v195, 182.
14. Davenport, H. W., *Am. J. Physiol.*, 1940, v129, 505.

Received August 25, 1952. P.S.E.B.M., 1952, v81.

Determination of Oxidative Enzymes and their Inhibitors by a Modified Chromatographic Method. (19811)

RUDOLF L. MAYER, MADELON R. GRIMM, AND FREDERICK C. KULL.
From the Microbiological Division, Ciba Pharmaceutical Products, Summit, N. J.

Present-day methods of detecting the action of melanin-forming enzymes and of evaluating the effect of enzyme inhibitors in these systems usually involve the incubation of enzyme-substrate mixtures and subsequent colorimetric determination of the melanin formed. Thus the activity of an anti-metabolite may be expressed as the decrease in melanin formation compared with that exhibited by control enzyme solutions. The sensitivity of this method depends not only upon enzyme concentration but also upon the diluent, the surface-volume ratio, temperature, etc. By this procedure, the anti-melanotic action of thiourea has been estimated at a dilution of approximately 1 x 10^{-4}(1-4). In view of the limitations of this test, a more sensitive method for the study of oxidase and anti-

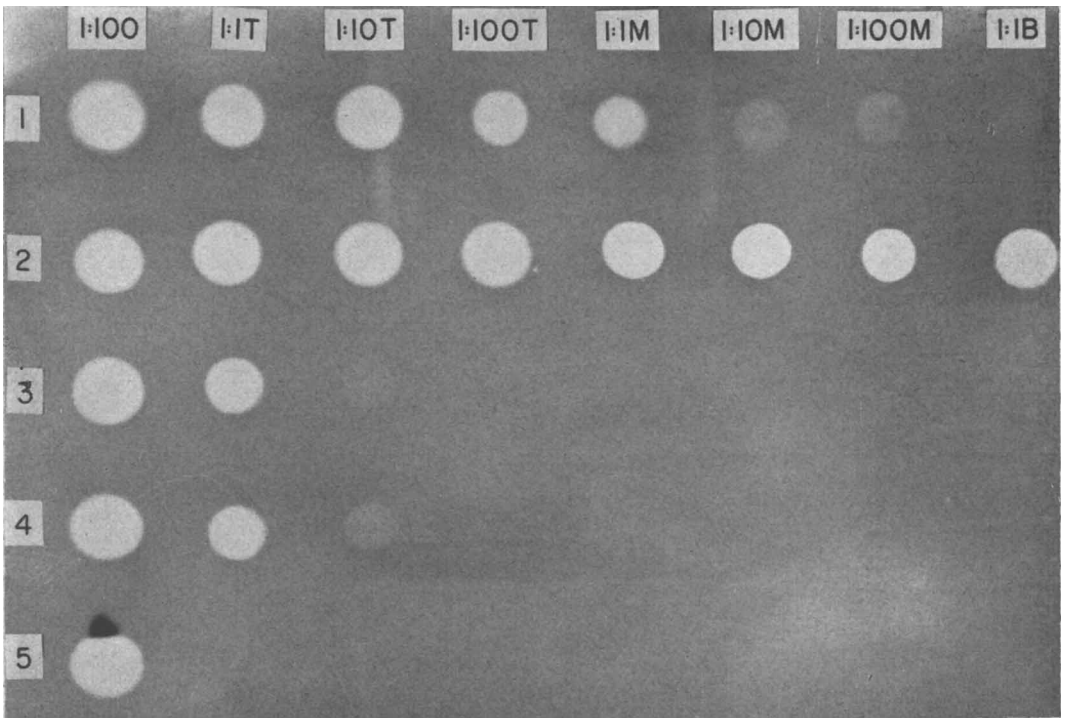

FIG. 1. Inhibition of phenol oxidase by (1) thiourea, (2) phenylthiourea, (3) Itrumil, (4) thiouracil, (5) propylthiouracil.