TABLE II. Effects of 5-Chlorouridine and Uridine on the Distribution of Phosphorus in the Acid-Insoluble Fractions of Minced One-day-old Mouse Brain. (μg of P per 100 mg of tissue).

Fraction		No addition	5-Chloro-uridine	Uridine	5-Chloro-uridine + uridine
LP	No virus avg*	35.7	34.4	38.5	38.3
	Range	35-36	34-35	36-40	38-39
	Virus avg	35.3	36.6	38.2	33.2
	Range	34-37	34-39	36-40	32-35
RNA	No virus avg	16.0	16.0	16.4	15.2
	Range	15-17	15-17	16-17	15-16
	Virus avg	19.9	15.8	20.2	18.3
	Range	19-21	15-17	20-21	18-19
DNA	No virus avg	12.4	14.5	13.0	12.5
	Range	11-13	13-15	12-14	12-13
	Virus avg	10.8	12.3	10.6	10.3
	Range	10-11	11-14	10-11	9-11
PIP	No virus avg	6.2	5.2	6.3	6.5
	Range	4-7	5-6	6-7	5-7
	Virus avg	5.9	6.1	4.8	5.8
	Range	5-7	5-7	4-5	5-6
RP	No virus avg	3.2	2.9	2.6	2.3
	Range	2-4	2-4	2-3	2-3
	Virus avg	3.7	2.7	2.5	2.6
	Range	3-6	2-3	2-3	2-3

* Avg of 3 experiments.

of RNA metabolism associated with virus propagation in this system.

Summary. 5-Chlorouridine inhibits the propagation of Theiler's GD VII virus and the uptake of $P^{32}O_4$ by the ribonucleic acid fraction of minced, 1-day-old mouse brain but had no effects on other tissue fractions studied. Uridine partially reverses the inhibiting effects of 5-chlorouridine on virus propagation and on the uptake of $P^{32}O_4$ by the ribonucleic acid fraction. Virus propagation has been shown to be associated both with an increased turnover and increased net amount of ribonucleic acid phosphorus.

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A Substance Occasionally Found as a Major Ninhydrin-Reacting Component of Urine.*† (18597)

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A ninhydrin-reacting substance has been observed frequently on 2-dimensional paper chromatograms prepared from the urines of many subjects, which appears not to match any of the 60 ninhydrin-reacting substances given in the "map of spots" by Dent(1), most of which were available for direct comparison, as well as a number of additional compounds available for checking. This unidentified compound falls between alanine and proline

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1. Dent, C. E., *Biochem. J.*, 1948, v43[2], 169.

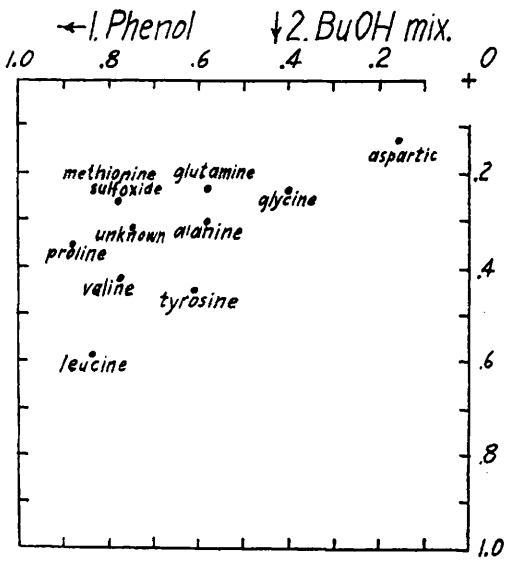

FIG. 1.

A 2-dimensional chromatogram showing the location of the unidentified substance and some common amino acids for orientation: Phenol saturated with water was used as the first chromatographing solvent and the upper layer of a mixture of tertiary butyl alcohol, secondary butyl alcohol, and water (1:5:5.6) as the second solvent.

when phenol is used as the chromatographing solvent, and between methionine sulfoxide and valine when the upper phase of either collidine, lutidine, and water (1:1:2) or tertiary butyl alcohol, secondary butyl alcohol, and water (1:5:5.6) is used as the solvent. Its position on a 2-dimensional chromatogram is shown in Fig. 1. It falls close to the position taken by α-amino-n-butyric acid on a 2-dimensional chromatogram, and judging from the frequency with which we have observed this compound on urine chromatograms (using 25 μl samples of first morning urine specimens from a large number of normal and pathological subjects) and the extreme rarity of any spot in the position of α-amino-n-butyric acid on these chromatograms, it is conceivable that others may have mistaken it for α-amino-n-butyric acid. The position of the unidentified compound in routine 2-dimensional chromatograms is close to the positions occupied by a number of known compounds, including sarcosine, pantonine, α-amino-ε-hydroxycaproic acid, α-amino-isobutyric acid, α-amino-n-butyric acid, γ-amino-n-butyric acid, σ-amino-n-valeric acid, and homocitrulline, but obvious differences can be shown with side by side comparison of R_f values in a wide variety of solvents. There remains the rather remote possibility that the unidentified substance in the urine might be a relatively stable salt, complex, or derivative of one of the known compounds with which it has been compared chromatographically or that it is a common substance previously known to be present in the urine but never reported as a ninhydrin-reacting constituent located on paper chromatograms. Attempts to isolate the substance from large volumes of urine in order that standard chemical procedures may be employed in its characterization have not as yet been successful but progress is being made in this direction.

A crude preparation of the unidentified compound has been obtained from 50 ml of urine by sectioning paper chromatograms. Urine from a leukemic patient who excreted the compound under investigation in high concentration was applied as a single line across the top of 10 sheets of filter paper, 22″ x 18½″. Phenol, with no ammonia in the atmosphere, was used as the chromatographing solvent, and when the boundaries were a few centimeters from the bottom the sheets were removed, air-dried, and examined in ultra violet light. All fluorescent bands were marked to serve as a guide for locating irregularities in the travel of bands on these overloaded papers. Two narrow strips, about one-half inch wide, were cut from each sheet of paper perpendicular to the line along which the urine was streaked originally and at about one-half of the distance from the center to each side of the sheet of paper. These strips were sprayed with ninhydrin in the usual fashion and the position of the unidentified compound could then be detected by the color reaction. A combination of the position of the ninhydrin-positive bands on the sprayed strips and the fluorescent bands were then used to locate the substance on the unsprayed sheets, and strips containing the unknown, together with some overlapping substances, were cut from each of the sheets of paper. A rectangular piece of filter paper, about 4 inches long, was sewed by machine to one end of each strip, and a triangular

FIG. 2.
Arrangement for collecting the substances from a strip of filter paper in a small volume of solvent: A strip of filter paper was sewed to the top for insertion in the solvent trough, and a triangular piece of filter paper was sewed to the opposite end of the paper, with threads left at the apex for tying to a glass collection bucket.

piece of filter paper was sewed to the opposite end of each strip. Long threads were left at the point of the triangular piece of paper, and these were tied to a small glass bucket, as shown in Fig. 2. The top of the strip was then supported in the trough of an all glass apparatus used for one-dimensional chromatography, and water was used as the solvent. The volume of water placed in the trough was small, so as to permit slow travel down the strip. When the water, which now contained substances washed from the strip, reached the triangular point of the paper, it followed down the threads and was collected in the bucket. When the bucket was full, the strip was removed and the sample collected from the bucket. The strip was allowed to dry, inserted in the trough again, and a second wash collected. Each strip cut from the original 10 papers was handled in this fashion, and the combined washings were pooled, streaked across eight large sheets of filter paper, and this time a mixture of tertiary butyl alcohol, secondary butyl alcohol, and water (1:5:5.6) was used as the chromatographing solvent. The sheets were then treated as previously, using fluorescent bands and small strips sprayed with ninhydrin as guide lines for cutting out the unidentified compound. The strips containing the unknown were sewed, buckets were attached, and the substance washed off with water. The pooled collections were streaked across one large sheet of filter paper which had been previously washed to remove the peptide contaminants(2), and chromatographed in phenol with ammonia present in the atmosphere. The unknown substance was removed as before, and streaked again on a sheet of paper previously washed, and chromatographed in collidine-lutidine-H_2O (1:1:2). The section of the paper corresponding to the compound in question was washed into the attached bucket, and this collection contained the unknown substance, with only faint traces of other ninhydrin-reacting compounds. After adjusting the volume to 7 ml, the intensity of the ninhydrin color produced by this fraction, using 5 μl for a 2-dimensional chromatogram, was roughly equivalent to that found with the common amino acids, using 5 μl of 0.01 N solutions. Probably around 10 mg of the material was obtained by the streaking technic, but although it was relatively free of other ninhydrin-reacting substances it showed no tendency to crystallize when subjected to the usual crystallization procedures.

This preparation has been used routinely as a marker for comparison with other amino acids available for study, for comparison with other urines showing the spot on 2-dimensional chromatograms, for following isolation procedures on a larger scale by other technics, and to determine some of the chemical properties of the compound.

The compound is stable to acid hydrolysis (6 N-HCl, 100° C, 24 hours). Its R_f value in phenol is not affected by ammonia or by hydrochloric acid in the atmosphere, nor is its rate of travel in phenol and collidine-lutidine affected by H_2O_2. The diazo test with the Pauly reagent(3) is negative, the bleaching reaction with potassium iodoplatinate for sulfur-containing amino acids described by Winegard and Toennies(4) appears to be negative, the benzidine-ammonium molybdate

2. Wynn, V., *Nature*, 1949, v164, 445.
3. Pauly, H., *Hoppe-Seyl. Z.*, 1904, v42, 508.
4. Winegard, H. M. and Toennies, G., *Science*, 1948, v108, 506.

color reaction for phosphate(5) is negative. The substance cannot be extracted with ether from a 0.3 N NaOH solution, which suggests it is not an amine. When the filter paper is brushed with basic cupric carbonate before the substance is chromatographed, using the technic described by Crumpler and Dent(6) as a distinctive test for α-amino acids, the ninhydrin test is not negative as for α-amino acids, but the ninhydrin color obtained is no longer the typical purple color, but has a brownish hue. This test has been carried out several times, using α-amino-n-butyric acid, γ-amino-n-butyric acid, and the unknown on the same strip. The α-amino acid consistently gave no color with ninhydrin, the γ-amino acid gave the typical color reaction, and the unknown gave an altered color. A somewhat similar reaction to copper dusting is shown by some amines and basic amino acids, but since the chromatographic behavior of the unidentified compound does not suggest a strongly basic nature, interpretation of the results of the copper test is equivocal.

Whatever the compound may be chemically, it is very interesting biologically. It has been observed occasionally in urines from some of our group of normal subjects composed of resident physicians, nurses, and laboratory personnel, and more frequently in urines from patients with certain pathological disorders. In many of the chromatograms, it is seen as a very light spot, but its presence is not restricted to cases of aminoaciduria or to highly concentrated urines. Occasionally it is seen to be the most intense spot on the chromatogram, and in a few cases, the only other ninhydrin-reacting substances detected were glycine, alanine and glutamine. It has been found in the urines of some of the patients examined with schizophrenia, epilepsy, various circulatory diseases, cerebral palsy, poliomyelitis, etc., but the highest concentrations have generally been observed in cases of malignancy. Chromatograms have been run on some patients with cancer over an extended period of time, and the excretion of the substance appears to be affected by some therapeutic regimes. In leukemia, for example, it may be reduced sharply in concentration or disappear with remission. Further data on the frequency of occurrence and the concentration of the compound in urine are being collected, and the results, together with information on the therapy and clinical state of individual subjects, will be reported.

Summary. An unidentified ninhydrin-reacting substance has been observed frequently on 2-dimensional paper chromatograms, prepared with urine from a wide variety of subjects, both normal and pathological. High concentrations are frequently found in the urine of patients with malignancy, and from results on some of the patients followed over an extended period of time, its concentration appears to be affected by certain therapeutic regimes. In leukemia, it may be reduced sharply in concentration, or disappear with a remission. A crude preparation has been obtained from urine by paper chromatography, and some of its properties investigated.

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5. Feigl, F., *Qualitative Analysis by Spot Tests*, Nordemann Publishing Company, Inc., New York (1937).

6. Crumpler, H. R. and Dent, C. E., *Nature*, 1949, v164, 441.