fore, a word of warning is indicated: it is probable that these analeptics should *not* be given a desperately poisoned human patient.

Since all the observations reported herewith have been on rats, it is not implied that the findings can be directly applied to the treatment of human poisoning. In the rat tests, the antidotes were given simultaneously with the EPN, such conditions would rarely be possible in industrial or accidental human exposures.

Summary. The administration of coramine and atropine together was considerably more effective in the treatment of EPN poisoned rats (both male and female) than was atropine alone.

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A Simple Method for the Determination of Urinary Amino Nitrogen.* (19192)

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Russell(1,2) adapted Folin's colorimetric technic(3) for the determination of blood amino nitrogen. According to Russell, this technic could not be used for urine because of interference by ammonia and uric acid. Therefore, it was the purpose of this investigation to adapt her procedure to the quantitative determination of urinary amino nitrogen.

Procedure. The reagents required, color reaction and technic were those described by Russell with the exception of Norit-A Decolorizing Carbon.

Removal of Ammonia: Volumes of urine ranging from 0.5 to 2.0 ml were pipetted into 200 ml Erlenmeyer flasks etched at the 100 ml mark. Approximately 75 ml of distilled water was added followed by 4 drops of 0.25% phenolphthalein in 95% ethanol. 0.1 N sodium hydroxide was added dropwise until the flask contents retained the pink color. This condition was reached at a pH between 9.0 and 9.5. The solution was boiled gently for 25 minutes. Base was added during the boiling process until the pink color no longer faded.

Removal of Uric Acid: After boiling off ammonia, the flask and its contents were cooled in an ice bath, and the solution acidified dropwise with 0.2 N hydrochloric acid until the pink color disappeared. Then one drop in excess was added. The solution was diluted to the 100 ml mark with distilled water and chilled in an ice bath for 10 minutes. Fifty mg of Norit-A Decolorizing Carbon was then added. The use of charcoal as an adsorptive agent for uric acid in urine was adapted from a procedure suggested by Bodansky(5).

After the addition of charcoal, the flask was stoppered, vigorously swirled for about a minute, and then allowed to stand at 5°C for a 2-hour period. At the end of this time, about 10 ml of the charcoal treated solution was transferred to a test tube and centri-

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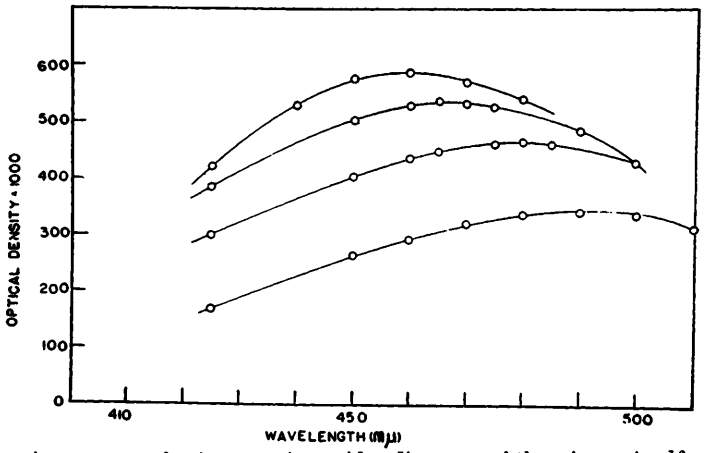

FIG. 1. Absorption spectra of urinary amino acid sodium-β-naphthoquinone-4-sulfonate complex.

fuged. After centrifugation, a 4 ml aliquot of the supernatant liquid was pipetted into the color development tube. The Russell color procedure followed from here.

Experimental. Absorption data were obtained for the color complex resulting when urinary amino compounds were coupled with the naphthoquinone reagent. Eight urine specimens were investigated in this manner.

In checking the technic for completeness of removal of ammonia, standard ammonium chloride solutions with an ammonium ion concentration approximately equal to that found in urine were subjected to the described boiling procedure, and analyzed for ammonia by the technic of Sobel(4).

In order to determine the optimum charcoal concentration for uric acid adsorption, carefully weighed quantities of charcoal were added to solutions of 3 mg per cent uric acid and known concentrations of standard glycine-glutamic acid mixture. The solutions were shaken, centrifuged, and the centrifugate analyzed for uric acid, by Brown's technic (6), and also for amino acid nitrogen. A glycine-glutamic acid standard curve was obtained for the range of 5 to 30 γ of amino acid nitrogen.

Results. Spectrophotometric absorption data were obtained for the urinary amino naphthoquinone complex in 8 different subjects. Representative spectra, showing the extremes in maximal absorption, are shown in Fig. 1. The lowest maximum absorption occurred at 460 mμ while the highest was at 490 mμ. The spectra gave a mean peak absorption of 475 mμ. In view of the broad absorption peaks observed and the mean absorption of 475 mμ, it was felt that the glycine-glutamic acid standard, which absorbed maximally at 470 mμ and was used by Russell for blood amino nitrogen, could be used for urinary determinations of amino nitrogen.

With this same standard solution, an essentially linear relationship between concentration and absorption was obtained for amino nitrogen concentrations within the range of 5 to 30 γ (Fig. 2).

Determinations for ammonia in boiled

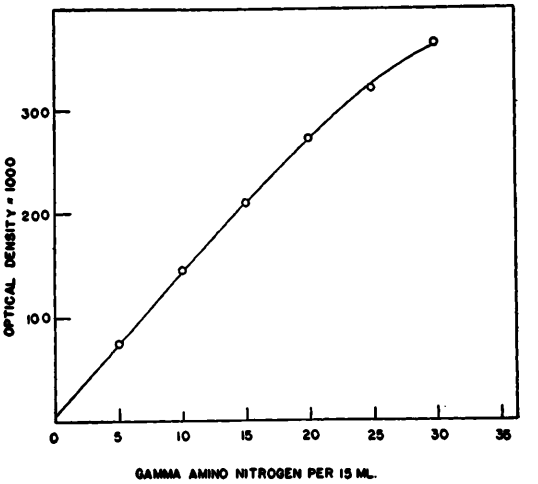

FIG. 2. Spectrophotometric determination of amino nitrogen in standard glycine-glutamic acid mixtures.

TABLE I. Recoveries of Amino Acid Nitrogen Added to Standard Uric Acid Solutions Containing Increasing Charcoal Concentrations. 125 γ amino N added to 25 ml of 3 mg % uric acid.

Charcoal added, mg	Uric acid recovered, mg	Amino N recovered, γ	%
13	.0	125	100
25	.0	125	100
44	.0	119	95
63	.0	116	92
125	.0	109	87
250	.0	98	78

TABLE II. Recoveries of Amino Acid Nitrogen When Increasing Amounts Are Added to Standard Uric Acid-Charcoal Mixtures. Amino N added to 25 ml of 3 mg % uric acid containing 13 mg charcoal.

Amino N added, γ	Uric acid recovered, mg	Amino N recovered, γ	%
50	.0	50	100
72	.0	72	100
100	.0	100	100
125	.0	125	100
147	.0	153	104
194	.0	200	103

standard ammonium chloride solutions demonstrated that boiling of the test (urine) solution at pH 9.0 to 9.5 for 25 minutes resulted in the complete removal of this substance.

The effect of activated charcoal on mixtures of uric and amino acids was studied. Bodansky reported quantitative uric acid adsorption from 430 ml of urine with 4 g of charcoal(5). In this present study with 3 mg % solution of uric acid, charcoal concentrations varying from 50 to 1,000 mg % were tested for completeness of uric acid adsorption. Table I shows that complete adsorption of uric acid occurred with all charcoal concentrations. This particular uric acid concentration was chosen since it is the highest that one would expect if urine were diluted at least 50 times as were the samples in this procedure. Non-charcoal treated urine gave amino nitrogen recoveries of about 113% of calculated. This error was in agreement with Russell.

Indiscriminate addition of charcoal resulted in low amino nitrogen recoveries. Table I shows that charcoal concentrations at and above 175 mg % resulted in losses of amino nitrogen of 5% or more. Thus, the optimal concentration of charcoal was found to be within the range of 50 to 100 mg %. It was found that this approximate quantity could be added without weighing. The amount of charcoal which covers the tip of a standard 180 mm nickel plated spatula is roughly 50 to 75 mg.

When increasing concentrations of amino nitrogen were treated with the optimal charcoal concentration, no loss of amino acids resulted (Table II). Data in Tables I and II are based on mean results of quadruplicate determinations with a precision of 1 to 2%.

Of 16 different urine specimens studied, a mean amino nitrogen recovery of 99% was obtained (Table III). The same accuracy of recovery held whether the standard glycine-glutamic acid mixture or a casein hydrolysate solution (10% amigen) was added to the urine. Analysis of the casein hydrolysate showed it to contain 740 mg % amino nitrogen with only very small daily variation in free amino content if properly stored and handled.

Since amigen contains considerable quantities of polypeptides, the hydrolysis of these substances to amino acids was a possibility. A control experiment revealed no increase in amino nitrogen when casein hydrolysate was subjected to the conditions of this determination.

When an abnormally high content of urinary protein was present, 5% sulfosalicylic acid was added to an equal volume of urine and centrifuged. The centrifugate was treated as were the other urine specimens. Recoveries of amino nitrogen from such urine yielded no loss of amino nitrogen (Table III No. 6).

Discussion. Of the several technics for determination of amino acid nitrogen, Van Slyke's titrimetric ninhydrin procedure is probably the most accurate and specific(7). However, urea is known to interfere in this procedure(8).

Of the colorimetric technics, Van Slyke notes that Folin's method as adapted by Russell is the most reliable(9). The latter author states that interference by uric acid and ammonia rendered her technic unsatisfactory for urinary determination of amino nitrogen although urea and polypeptides did not interfere.. The technic described in this paper provides simple methods for eliminating

TABLE III. Recoveries of Standard Glycine-Glutamic Acid Mixtures (S) and Amino Acids of Casein Hydrolysate (C) Added to Urine.

Urine specimen No.	Volume of urine diluted to 100 ml, ml	Amino N in urine, mg %	Amino N added to urine, γ	Amino N in urine containing added N, mg %	Recovery of added amino N, %
1	2	24.2	250 (S)	40	98
2	1.50	18.5	258 (S)	34.8	95
3	1.50	34.8	258 (S)	52.7	103
4	1.50	43	250 (S)	59.1	99
5	.50	30	270 (S)	82.5	98
6	1	12.2	245 (S)	37	101
7	1	43.3	377 (C)	79.5	98
8	1	48.3	377 (C)	87.5	102
9	1	19.5	237 (S)	83.3	100
10	1	37	313 (C)	69.3	101
11	1	38.2	313 (C)	68.3	98
12	1	16.3	285 (C)	43	96
13	2	8.8	260 (S)	21.9	100
14	1.50	11	238 (S)	26.7	100
15	1	9.5	260 (S)	35	99
16	1	27	260 (S)	52.5	100
				Mean recovery	99%

No. 1-12, casual and fasting specimens; No. 13-16, 24 hr specimens.

these complicating substances, and it is possible that the procedure might be adapted to the needs of the clinical chemical laboratory.

Conclusion. 1. Russell's colorimetric procedure for the determination of blood amino nitrogen has been adapted for urine. 2. A simple method has been described for removing the interfering substances, ammonia and uric acid, without altering the precision and accuracy of the colorimetric technic. 3. Recoveries of urinary amino nitrogen were attained with a mean value of 99%.

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Calcification. IV. Influence of Strontium and Magnesium Ions on Calcification *in vitro*.* (19193)

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It was reported earlier by one of the authors that strontium in small amounts inhibits the calcification of rachitic cartilage *in vitro* (1,2). In attempting to repeat this experiment, Marks and Shorr(3) were unable to detect such inhibition. In order to resolve this discrepancy, the subject was further investigated. As will be shown below, the results indicate the importance of magnesium ions in the inhibition of the calcifying

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