The Use of Urograph for the Determination of Urea Nitrogen Concentration in Serum and Plasma

J. E. LOGAN, Ph.D., *Ottawa, Ont.*

ABSTRACT

The accuracy and precision of Urograph, a commercial preparation of chromatography papers designed for the determination of urea nitrogen in plasma and serum, were assessed. Results of duplicate determinations were compared with those of two acceptable quantitative procedures, (1) automated diacetyl monoxime and (2) modified Van Slyke and Cullen analysis. Values for serum specimens obtained by Urograph were significantly higher than those found by the Autoanalyzer method. The confidence limits for the Urograph procedure ranged from 12.5% for plasma to 26.6% for sera, whereas the corresponding values were 6.9% and 7.0% for Autoanalyzer. This lack of precision with Urograph appeared to be due mainly to the presence of a few strips in which only partial migration occurred. The papers yielded low values following 8½ months of storage in the refrigerator; the test was temperature-sensitive.

UROGRAPH* is the commercial name given to urea nitrogen chromatography papers manufactured as "an accurate, simple, reproducible micro method for the determination of urea nitrogen in either serum or plasma." These papers are treated with controlled amounts of reagents similar to those used in the Conway micro-diffusion procedure[1] for determination of blood urea nitrogen. The lowest band on the paper strip contains an area of phosphate-buffered urease, the next potassium carbonate, and the highest an indicator area of bromcresol green in tartaric acid. When the tip of that portion of the strip impregnated with the reagents is immersed in 0.1 ml. of serum or plasma in a 10 x 75 mm. test tube, migration of the specimen up the strip takes place. It first contacts the urease where urea is hydrolyzed to form an ammonium salt which is then converted to free ammonia by the potassium carbonate in the next area. A Krylon plastic barrier just beyond this area prevents further migration of the specimen into the indicator. With the release of ammonia over a 30-minute test period, the level of the gas builds up in the bottom of the tube and when it reaches the indicator level a colour change occurs on reaction with the tartaric acid. The height of the colour on the indicator area is proportional to the amount of ammonia released.

Since the determination of urea nitrogen in the hospital laboratory might be carried out very simply, rapidly and at relatively low cost by means of Urograph, it was of interest to determine the accuracy and precision of results which could be obtained by this procedure. As an assessment of accuracy, all results obtained with Urograph were compared with the diacetyl monoxime method as adapted to the Autoanalyzer,[2] and some results obtained with the modified Van Slyke and Cullen method.[3] Previous work in our laboratory has indicated that results obtained by the Autoanalyzer procedure agree well with those of the modified Van Slyke and Cullen method. All the determinations were run in duplicate to ascertain the precision of the method. In addition, some observations were made on the effect of temperature on the test and the stability of the product on storage in the refrigerator.

METHODS

The instructions and precautions for using Urograph as supplied by the manufacturer were followed carefully. The migration of a 0.1-ml. specimen was carried out on each occasion at a temperature within the acceptable range of 20°-26° C., and the levels were read at 30±1 minutes. Values were determined from the strips by means of the caliper supplied by the manufacturer. This caliper was set at the labelled value of a Versatol-A control serum and at the level reached by this serum in the migratory test. The control sera used had previously been found to be accurate within ±5% of the stated values when determined by the Autoanalyzer and modified Van Slyke and Cullen procedures.

The modified Van Slyke and Cullen procedure as detailed in the Department of National Health and Welfare's Manual of Clinical Chemistry[3] was followed and the diacetyl monoxime procedure was carried out as described in the Technicon Autoanalyzer Manual.[2] For studies of the effect of temperature on migration, the temperature was controlled to within ±0.5° C. by means of a water bath.

RESULTS

The results of a comparison of urea nitrogen values obtained by Urograph and the Autoanalyzer on 40 blood sera from hospital patients are shown in Table I. All specimens were run in duplicate and the comparison is made on the average of the duplicates. The data are grouped into four ranges of differences and the number of specimens which fall into these ranges are recorded. It will be noted

From the Clinical Laboratories, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Ont.
*Trademark for product of Warner-Chilcott Laboratories, Toronto.

Canad. Med. Ass. J.
Aug. 24, 1963, vol. 89

TABLE I.—DIFFERENCES OF UROGRAPH VERSUS AUTOANALYZER IN MEAN VALUES ON 40 HOSPITAL SERA (RESULTS ARE AVERAGES OF DUPLICATES)

Urograph	*Mean values Autoanalyzer*	*Mean difference*
20.8 mg./ml.....	18.4	+2.4 (P < 0.001)
		No. of specimens
Range of differences (±mg./100 ml.)		*Higher (compared to Autoanalyzer)* / *Lower (compared to Autoanalyzer)*
0 - 0.9		2 / 1
1.0 - 2.9		12 / 1
3.0 - 4.9		14 / 0
5.0 and over		7 / 3
Totals		35 / 5

that the mean of all values as determined by Urograph is significantly higher than that determined by the Autoanalyzer (P <0.001). Of the 40 specimens, 35 of the values obtained by the Urograph method were higher. Twenty-four Urograph results differed by 3 mg. % or more from those obtained by the automated procedure. The range of differences for these sera was from minus 5.5 mg. % to plus 6.4 mg. %.

TABLE II.—DIFFERENCES OF UROGRAPH VERSUS AUTOANALYZER IN MEAN VALUES ON 48 HOSPITAL PLASMAS (RESULTS ARE AVERAGES OF DUPLICATES)

Urograph	*Mean values Autoanalyzer*	*Mean difference*
22.0 mg./100 ml.....	21.8	+0.23 (P > 0.7)
		No. of specimens
Range of differences (±mg./100 ml.)		*Higher (compared to Autoanalyzer)* / *Lower (compared to Autoanalyzer)*
0 - 0.9		3 / 2
1.0 - 2.9		18 / 4
3.0 - 4.9		13 / 3
5.0 and over		1 / 4
Total		35 / 13

A similar comparison is shown in Table II for 48 blood plasma specimens. No significant difference was found between the mean values obtained by the two methods. However, 35 of the 48 values obtained by Urograph were higher than those obtained by the Autoanalyzer procedure. Three Urograph results were very low, i.e. 9.5, 10 and 30 mg. % lower than those obtained by the Autoanalyzer, thus markedly lowering the mean value for the group. It will be noted that 21 of the 48 comparative values differ by 3 mg. % or more. The entire range was from minus 30 mg. % to plus 5.5 mg. %.

TABLE III.—DIFFERENCE OF UROGRAPH VERSUS OTHER METHODS IN MEAN VALUES ON 16 COMMERCIAL CONTROL SERA (RESULTS ARE AVERAGES OF DUPLICATES)

Urograph	*Mean values Autoanalyzer*	*Van Slyke and Cullen*
24.7 mg./100 ml.....	27.1 mg./100 ml.	27.6 mg./100 ml.
	Mean differences Uro. vs. Van Slyke	
Uro. vs. A.A.		*A.A. vs. Van Slyke*
−2.4 (P > 0.1).....	−2.9 (P> 0.05)	−0.54 (P > 0.1)
	Urograph versus Autoanalyzer	
		No. of specimens
Range of differences (±mg./100 ml.)		*Higher* / *Lower*
0 - 0.9		3 / 0
1.0 - 2.9		2 / 4
3.0 - 4.9		1 / 0
5.0 and over		1 / 5
Totals		7 / 9

In Table III comparisons were made of three methods, Urograph, Autoanalyzer, and modified Van Slyke and Cullen, in determining urea nitrogen on commercial sera. Sixteen different sera, normal and abnormal, from five manufacturers were used. There was no statistically significant difference in mean values obtained by the Urograph and Autoanalyzer procedures, although the Urograph mean value was 2.4 mg. % lower. There was closer agreement in the values obtained by the Autoanalyzer and the modified Van Slyke and Cullen methods. Results for seven of the 16 specimens analyzed by Urograph and by the Analyzer differed by 3 mg. % or more. Urograph values ranged from 19.9 mg. % lower to 8.1 mg. % higher in this comparison.

The precision of the three methods is compared on three different types of specimen in Table IV.

TABLE IV.—PRECISION OF METHODS

Type of specimen	Percentage confidence limits*: *Urograph*	*Autoanalyzer*	*Van Slyke and Cullen*
Blood sera...	± 26.6% (39)	± 7.0% (39)	± 8.5% (9)
Blood plasmas...	± 12.5% (52)	± 6.9% (28)	——
Commercial sera.....	± 24.5% (36)	± 4.3% (33)	± 5.7% (17)

*Calculated as: $\frac{\pm 3 \times \text{standard deviation of duplicates} \times 100}{\text{Mean urea nitrogen values}}$

The percentage confidence limits are calculated by the method of Copeland[4] and it will be noted that they are considerably greater for the Urograph method than for either the Autoanalyzer or the modified Van Slyke and Cullen methods. In the case of the Urograph procedure six of 39 sera produced duplicate values differing by 3 mg. % or more, and among the plasma samples none of the duplicates exceeded differences of 4 mg. %. For commercial control sera, eight of 36 pairs of duplicates differed by 3 mg. % or more.

Table V shows the comparison of results on 25 commercial and patients' sera using a batch of Urograph that had been stored in the refrigerator for 8½ months, with results obtained using Urograph that had been stored only about 1½ months before the estimations were performed. Significantly lower values were obtained with the older lot of Urograph.

TABLE V.—STABILITY OF UROGRAPH MEAN VALUES (25 COMMERCIAL AND HOSPITAL SERA)

Old Urograph	*Fresh Urograph*	*Mean difference*
21.7 mg./100 ml.....	24.4	−2.7 (P < 0.02)

Range of differences (±mg./100 ml.)	*No of specimens*	
	Higher (compared to fresh Urograph)	*Lower (compared to fresh Urograph)*
0 - 0.9	4	6
1.0 - 2.9	2	4
3.0 - 4.9	1	3
5.0 and over	0	5
Totals	7	18

Triplicate determinations were performed on two types of commercial sera when migration was carried out at three water-bath-controlled temperatures: 20° C., 25° C. and 30° C. In Table VI, it

TABLE VI.—EFFECT OF TEMPERATURE DURING MIGRATION ON UROGRAPH VALUES OBTAINED WITH COMMERCIAL CONTROL SERA

Specimen	*20° C.*	*25° C.*	*30° C.*
Commercial serum A.....	25 mg./100 ml.	23	35
	27	26	37
	28	28	37
Commercial serum B.....	23	25	27
	24	27	27
	25	28	27

will be seen that with commercial serum A, a reconstituted type, the values at 20° C. and 25° C. were comparable, whereas at 30° C. the values were much higher. On the other hand, with a liquid preparation B, the values at 25° C. and 30° C. were comparable, while those obtained at 20° C. were a little lower.

DISCUSSION

An attempt to assess the accuracy of Urograph in the determination of urea nitrogen has yielded variable results. In the case of serum specimens, the mean values obtained by this method were significantly higher than those provided by the Autoanalyzer, but no significant difference between these two methods was obtained for plasma and commercial control serum. The lack of a significant difference would appear to be due to the occurrence of Urograph values which were 10 mg. % or more lower than the corresponding Autoanalyzer values. It may be concluded that Urograph is not an accurate method of measuring urea nitrogen, since it yielded values higher than the Autoanalyzer with the sera obtained from hospital patients. Martin and Seibel[5] compared Urograph values on 10 consecutive samples only, received in the routine biochemistry section, with those obtained by the direct colorimetric acid-diacetyl method of Natelson, Scott and Beffa[6] and found no significant difference in the mean values of the group. Individual differences ranged from 2.2 mg. % lower to 2.2 mg. % higher by the Urograph procedure.

Our results have shown that the Urograph method lacks the precision of either the Autoanalyzer or modified Van Slyke and Cullen methods. If one calculates the acceptable error of a method as one-quarter of the range of normal values divided by the mean value of that range, and multiplied by 100, one obtains for blood urea nitrogen the following percentage error:

$$\frac{\frac{1}{4}\ (18\text{-}10)\ \times\ 100}{14} = 14.3\%$$

where a normal range of 10-18 mg./100 ml. is used. From this it will be seen that the precision of the Urograph method becomes acceptable only in the case of the analyses on the blood plasma, and there the error is considerably greater than that encountered with the Autoanalyzer procedure. A careful examination of the Urograph data indicates that in many cases the variation between duplicates is not great (i.e. 3 mg. % or less), but the occasional extremely poor duplication (as high as 12 mg. % difference) has been responsible for the high percentage confidence limits obtained in the analyses of hospital patients' and commercial control sera. In the patients' sera where duplicates for four specimens differed by 5 mg. % or more, one value was found to be in the normal range while its duplicate fell in the abnormal range in the case of three of these specimens. This type of result could lead to an incorrect finding for the individual patient, particularly if the test were not run in duplicate. The running of duplicates would at least cast suspicion on the results. Although Martin and Seibel[5] report that the reproducibility of the Urograph is excellent, the determination of percentage confidence limits on their data by the method of Copeland[4] would not confirm this. In our hands, we have obtained values of 19.3% and 14.0% for the normal and abnormal control sera, respectively.

Our results have led us to conclude that the poor precision obtained with Urograph is due to the failure of a few strips to give reproducible migratory responses. It would appear that in the case of these strips only a partial migration takes place, possibly owing to improper impregnation of reagents or some other defect in the manufacturing process. In fact, in our total experience with over 400 strips, we encountered two strips where no migration at all occurred. This level of precision would not justify use of these chromatography papers as a quantitative procedure for determination of urea nitrogen. On the other hand, they might serve as a screening device, provided that the determination was carried out in duplicate on each occasion.

Our results also indicate that Urograph is not stable for a very long period even when stored in the refrigerator according to the manufacturer's instructions. Significantly lower values were obtained when a batch that had been stored for 8½ months prior to use was compared with one stored only for 1½ months.

As indicated by the manufacturer, the reaction occurring in Urograph is sensitive to temperature. In our results, a marked increase in migration at 30° C. was experienced in the case of a reconstituted commercial control serum, whereas in the case of a liquid commercial serum no change was observed from the value obtained at 25° C. In the latter instance, the value at 20° C. seemed to be lower than when the experiment was run at 25° C. or 30° C. We have not determined the cause of this apparent difference in migratory response between the two types of sera.

SUMMARY

The comparison of urea nitrogen values determined by Urograph with those obtained using the Autoanalyzer and modified Van Slyke and Cullen techniques has indicated that the Urograph method is not accurate. It was also found to lack that precision necessary to achieve results within acceptable limits of error for the blood urea nitrogen method. The lack of precision appeared to be due to the presence of a few strips in which only partial migration occurred. Results following 8½ months' storage in the refrigerator were not reliable. The migration process was found to be quite temperature-sensitive and may be partly dependent on the type of specimen tested.

The author wishes to express his thanks to Dr. R. H. Allen, Chief, Clinical Laboratories, Laboratory of Hygiene, for his suggestions and advice, and to Mrs. Daisy Taada for skilful technical assistance. He is also indebted to Dr. H. Richter, Ottawa Civic Hospital, for providing serum and plasma specimens, and to the Warner-Chilcott Co., Toronto, for a generous supply of Urograph papers.

REFERENCES

1. CONWAY, E. J.: Micro-diffusion analysis and volumetric error, Crosby Lockwood & Son, Ltd., London, 1947.
2. Technicon Instruments Corporation: Technicon autc analyzer manual, Chauncey, New York, 1959.
3. Canada, Department of National Health and Welfare, Laboratory of Hygiene: A manual of clinical chemistry, Ottawa, 1955.
4. COPELAND, B. E.: *Amer. J. Clin. Path.*, **27**: 551, 1957.
5. MARTIN, G. M. AND SEIBEL, L.: *Canad. Med. Ass. J.*, **83**: 1214, 1960.
6. NATELSON, S., SCOTT, M. L. AND BEFFA, C.: *Amer. J. Clin. Path.*, **21**: 275, 1951.

GENERAL PRACTICE

Current Concepts in Dermatology: Part I

ROBERT JACKSON, M.D., F.R.C.P.[C],* *Ottawa, Ontario*

ABSTRACT

Some of the more recent advances and newer concepts about certain dermatological conditions are presented. Untoward reactions to drugs, whether to systemic or topical agents, are discussed in detail; emphasis is placed on their frequency and on the diversity of clinical findings. In the management of certain skin tumours (hemangiomas and melanocytic nevi) it is essential that the natural history of the lesion be considered. It is generally agreed that the answer to the problem of staphylococcal infection of the skin will be found in the host rather than the staphylococcus. Keratoacanthoma is a pseudocarcinoma of unknown etiology and sometimes gives rise to considerable difficulty in diagnosis. Diffuse alopecia in women is now a common dermatological problem, the cause of which is unknown. The hair loss associated with it is not permanent.

THE review which follows has been prepared to acquaint the practising physician with recent clinical and therapeutic developments in the field of dermatology, with emphasis on the more commonly encountered disorders of the skin.† For convenience, it has been divided into three parts. Part I deals primarily with specific cutaneous disorders; Part II presents a consideration of cutaneous manifestations of systemic disease; and Part III is concerned with the use of radiotherapy and corticosteroids in dermatology.

PART I

Diffuse Alopecia in Women

Nothing brings a woman to her doctor more quickly than the fear of going bald. Alopecia areata, with its single or multiple circular bald patches and with the exclamation point hairs at the periphery, has been known for many years. Its causes and treatment remain as enigmatic as ever. In addition, a new entity has been described recently; Sulzberger, Witten and Kopf[1] drew attention to a condition in middle-aged women called diffuse non-scarring alopecia. Oily seborrhea may be found

*Dermatologist, Ottawa Civic Hospital.
†For those interested in the basic sciences as they apply to dermatology, "Progress in the Biological Sciences in Relation to Dermatology", edited by Arthur Rook, Cambridge University Press, 1960, is recommended as a comprehensive reference text.