THE EXCRETION OF BIURET IN THE URINE OF SHEEP FED BIURET

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INTRODUCTION

In a nitrogen balance test with steers receiving biuret, Hatfield, Forbes, Neumann & Garrigus (1955), reported that an average of 12·8 gm " apparent biuret " was excreted in the urine per day.   As the intake was 90 gm this represented 14·2 per cent of the intake.   The method of determining the biuret in the urine was not reported.

During toxicity trials on sheep in which 250 gm biuret was given in one dose, Clark, Barrett & Kellerman (1963) noted that the urine developed a heavy crystalline deposit on standing.   A sample of this deposit was submitted to the laboratories of African Explosives and Chemical Industries, Ltd., and was reported on as being " practically pure biuret ".

As investigations into the use of biuret as a non-protein nitrogen supplement were being initiated in these laboratories it was decided to investigate this apparent loss further.   Since no method for the quantitative determination of biuret was available, a method had to be developed.

METHODS

The determination of the solubility of biuret in sodium hydroxide solutions

The biuret used was 98 per cent pure.

The first difficulty arose from the low solubility of biuret in water (1·54[15]).

Biuret was known to be more soluble in sodium hydroxide solutions but accurate figures were not available and had to be determined.   Various concentrations of sodium hydroxide were made up as indicated in Table 1.   Biuret was added to each sample progressively until saturated solutions at room temperature were obtained. The samples were allowed to stand until all suspended particles had settled out. From each sample, 10 ml of saturated solution was placed into clean, dry, weighed, evaporating dishes.   The dish and contents were weighed immediately and then placed in a drying oven at 50° C.   After evaporation of the water, the temperature was raised to 105° C for two hours.   The dishes were then transferred to a desiccator to cool and were then weighed.   From the weights so obtained, the solubility of biuret in the various initial known concentrations of NaOH was calculated.   All solutions were made up in triplicate and the averages taken as the final result.   The results thus obtained were corrected for the maximum amount of Na_2CO_3 which could be formed from the NaOH present in each sample during the entire operation.   The results are presented in Table 1.

TABLE 1.—*The solubility of biuret in NaOH solutions*

Normality of NaOH	Weight of Residue (a)	Weight of Na_2CO_3 formed (b)	Weight of dissolved Biuret (Anhyd.) (a−b)	Corrected weight of Biuret (Crystalline)	Solubility of Biuret
	gm	gm	gm	gm	%
0·10	0·3083	0·0530	0·2553	0·300	3·00
0·15	0·3817	0·0795	0·3022	0·355	3·55
0·20	0·4167	0·1060	0·3107	0·365	3·65
0·25	0·4832	0·1325	0·3507	0·412	4·12
0·50	0·7384	0·2650	0·4734	0·556	5·56
0·75	0·9745	0·3975	0·5770	0·678	6·78
1·00	1·2520	0·5300	0·7220	0·848	8·48

As will be seen, biuret was found to be 8·48 per cent (w.w.) soluble in N NaOH. More concentrated solutions of NaOH were not tried, as it was noted that when biuret was added to such solutions decomposition of the biuret was indicated by a distinct smell of ammonia.

The quantitative determination of biuret in urine

The method selected for the quantitative determination of biuret in urine was based on the well known copper biuret reaction, Weichselbaum's reagent, as described by King & Wootten (1959) being used. As the method is colorimetric, a technique for decolorizing the urine had to be evolved.

From the findings regarding the solubility of biuret, it was decided that the first step should be the addition of an equal part of 2N NaOH to a urine sample. This would give an initial concentration of N NaOH which would ensure the biuret being dissolved.

Upon addition of 2N NaOH (1 : 1 with urine) slight precipitation of organic matter occurred. The complete precipitation of this organic matter was accomplished by using $ZnSO_4$. However, the normal colour of urine remained unaffected (urochrome, urobilin, indigoid pigments and other colouring matter). Complete decolorization was achieved with activated charcoal.

Experiments were carried out to determine the optimum proportions of supernatant and Weichselbaum's reagent. It was found that 2 ml supernatant +7 ml Weichselbaum's reagent gave a white, cloudy precipitate, which dissolved on the addition of 2 ml, 10N NaOH, to give the typical colour reaction.

Investigation showed that if the cloudy precipitate was centrifuged out and the supernatant drawn off, no measurable colour developed in the supernatant. However, upon dissolving the precipitate in 2 ml 10N NaOH and adding this solution to the supernatant, the typical colour reaction occurred. Samples so treated gave the same colorimeter reading (±0·1 division) as samples of the same biuret concentration which were uncentrifuged and to which 2 ml 10N NaOH had been added.

On these findings, the following method was adopted:—

For each urine sample:—

(i) 10 ml urine+10 ml 2N NaOH. Mix well. Stand 10 minutes.

(ii) 10 ml of mixture+1·5 to 2 gm zinc sulphate. Shake very well and centrifuge at ±3000 rev. per min.

(iii) Draw off supernatant and add ±1 gm activated charcoal. Shake intermittently for 10 min. Centrifuge for 5 min.

(iv) To 2 ml of this supernatant, add 7 ml Weichselbaum's biuret reagent and 2 ml 10N NaOH. Shake well and stand for 30 min.

(v) Read colour formed against the blank in a colorimeter using filter " Ilford No. 625 " (540 mμ) or equivalent. From the standard curve, the biuret concentration may be determined.

Blank:—

2 ml water (dist.)+7 ml Weichselbaum's reagent+2 ml 10N NaOH. Mix well.

Using the final routine method adopted, a standard curve was constructed from urine samples of known biuret concentration. Each determination for the standard curve was done in triplicate, and the mean of the three readings was plotted.

Three urine samples containing no biuret were treated as above, and each gave a colorimeter reading of 0·0 against the blank.

The results are shown in Table 2.

TABLE 2.—*Calibration figures: Urinary biuret concentration and colorimeter readings*

Sample No.	Weight of Biuret per 100 ml in mg	Colorimeter	Mean of each group of three readings
Blank—			
(i).........	0·0	0·0	Blanks (i); (ii) and (iii) - 0·0
(ii).........	0·0	0·0	
(iii).........	0·0	0·0	
A..............	303·0	0·30	A; B and C=0·29
B..............	303·0	0·28	
C..............	303·0	0·28	
D..............	665·0	0·65	D; E and F=0·63
E..............	665·0	0·55	
F..............	665·0	0·69	
G..............	998·0	0·96	G and I=0·98 (test tube H was broken)
H..............	998·0	—	
I..............	998·0	0·99	
J..............	1351·5	1·31	J; K and L=1·32
K..............	1351·5	1·32	
L..............	1351·5	1·43	
M..............	1703·0	1·63	M; N and O=1·63
N..............	1703·0	1·64	
O..............	1703·0	1·62	

The mean error of the method is ±2·001%

As will be seen, the readings obeyed the Beer-Lambert law within the concentrations used and the reproducibility was very good.

In order to determine whether ammonia, which could be expected to be present in variable amounts in the alkaline urine samples, interfered with the determination, the following test was carried out. The ammonia present in urine samples collected under the prevailing conditions was determined by aeration into a boric acid—indicator solution and titration with acid. The amounts found varied from traces to some 50 mg per cent. Approximately 1 per cent biuret was then added to a bulk sample of urine which on test was found to contain 37 mg per cent ammonia. The solution was divided into four aliquots and ammonia added to three of these to bring the final concentrations to 37, 60, 76 and 112 mg per cent respectively. After following the above procedure the colorimetric readings were found to be $1 \cdot 19$, $1 \cdot 19$, $1 \cdot 17$ and $1 \cdot 18$ respectively. Furthermore no ammonia could be detected in any of the supernatants after step (iii) of the method described. These findings, together with the fact that all biuret-free urine samples gave a reading of $0 \cdot 0$ against a water blank proved that varying amounts of ammonia present did not affect the final reading.

The determination of biuret excreted in the urine of sheep fed biuret

Two Merino sheep (wethers), each with a permanent rumen fistula were placed in separate metabolism cages. Each animal was fitted with a faeces-collection bag to prevent contamination of the urine. Both sheep were on a diet of teff-hay and water *ad lib.* plus 5 gm stock salt and 5 gm bone meal each per day.

Each animal was dosed 10 gm biuret via fistula per day for five days. During this base-line period, the urine was discarded and the collection bottles were thoroughly washed out each day at $9 \cdot 00$ a.m. On the fifth day, the urine passed during the preceding 24 hour period was collected, the volume measured, and the biuret concentration determined. From these figures, the daily biuret excretion via the urine from a daily dose of 10 gm was calculated.

In this manner, the daily excretion from doses of 10 gm, 15 gm, 30 gm and 50 gm per day was determined. A second series was run, using the same rates of dosage. A period of two weeks was allowed between the end of the first series (50 gm dose) and the beginning of the second series (10 gm dose).

TABLE 3.—*Daily urinary excretion of biuret*

Dose	Sheep No. 1			Sheep No. 2		
	Excretion (first series)	Excretion (second series)	Mean excretion	Excretion (first series)	Excretion (second series)	Mean excretion
gm	gm	gm	gm	gm	gm	gm
10	2·81	4·20	3·50	2·42	3·20	2·81
15	5·2	3·96	4·58	2·34	3·21	2·78
30	9·06	6·00	7·53	7·25	6·30	6·78
50	21·99	8·47	15·23	11·08	7·07	9·08

DISCUSSION

The results show that some 20 to 30 per cent of the biuret dosed was excreted as such in the urine. This figure is higher than that reported by Hatfield *et al.* in 1955 i.e. 14·2 per cent. In our trials the biuret was administered as a single dose, which would cause a very much higher temporary concentration in the rumen than when it was taken in more gradually with the food. Such conditions would mitigate against its optimal utilization by the ruminal organisms and cause more rapid absorption and excretion. The figures reported in this paper therefore probably do not represent those that can be expected in practical biuret feeding.

The fact that a certain proportion of the dietary biuret is excreted in the urine cannot be taken as an indication that the utilization of biuret will be less efficient than that of urea. It is reasonable to assume that the same proces takes place in urea feeding but the origin of urinary urea cannot be identified.

REFERENCES

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