visible light from the source. Vertical partitions (12) separate off a central compartment of the box and serve as supports for the three mirrors which it contains. Two of these (13) lie at right angles to each other in vertical planes, while the third (14) makes an angle of 45° with the horizontal.

Fluorescent light from the two test tubes enters the central compartment through square apertures (15); it is reflected by the vertical mirrors on to the oblique mirror and thence upwards into the eyepiece (16) through a hole in the top of the box. The base of the eyepiece tube contains a filter (17) which cuts off practically all light except the wave-lengths produced by the fluorescence of porphyrin.

Interposed between the standard and the aperture (15) are the two glass screens (18); the dark end of one is above and of the other below, so that where they overlap they produce a uniformly shaded field. The screens are mounted in slides (19) which can move up or down a vertical guide (20). Each is held at the desired level by a pin (21) which enters a spiral groove in a vertical cylinder (22). The spiral is in one case right-handed and in the other left-handed. The two cylinders are fixed one above the other to a spindle (23) carried in bearings in the top and base of the box; a knurled head and a dial divided into a hundred divisions are mounted on the spindle above the top of the box. The spiral groove is of such a pitch that the whole range of movement of the slides is achieved by slightly less than one complete revolution of the spindle, and, as the grooves are right- and left-handed, equal and opposite movements are given to the two slides by a given degree of rotation.

The slides are of such a length that their less dense ends are separated by a gap of about $\frac{1}{4}$ in. when the dial knob is at zero, so permitting unobstructed light to pass in this position from the right-hand tube to the mirror for comparison with the tube on the left. This arrangement has been found convenient for certain determinations in which a blank had to be evaluated.

The inside metal work of the apparatus is painted black and the tube of the eyepiece is lined internally with matt surfaced black paper.

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The Utilization of Urea in the Bovine Rumen. 1. Methods of Analysis of the Rumen Ingesta and Preliminary Experiments *in vivo*

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Some evidence has accumulated during the past 40 years to suggest that ruminants can utilize simple non-protein nitrogenous compounds such as urea when these compounds replace a proportion of the protein normally present in the diet. The work, much of which is highly controversial, has been reviewed recently by Krebs [1937], and also by Owen [1941]. Since these reviews appeared, publications by Owen, Smith & Wright [1941; 1943] in this country and by Hart, Bohstedt, Deobald & Wegner [1939] in America have shown that urea undoubtedly has some definite nutritive value when fed to ruminants in suitable amounts and in appropriate mixtures. It has been suggested that certain micro-organisms which multiply in the rumen build up their own body protein from this simple form of nitrogen. These micro-organisms then pass further along the alimentary tract where their protein is digested with the ordinary protein of the diet. The work here described was undertaken to test the validity of this theory.

A steer with a large rumen fistula was employed and in the original plans, made in 1938, it was hoped to use this animal, in the first place for *in*

vivo experiments in which urea would be fed and any changes occurring in the N distribution in the rumen ingesta would be observed, and secondly for obtaining samples of the rumen contents for *in vitro* incubations with urea.

It was first necessary to establish reliable methods for the accurate determination of non-protein nitrogen (N.P.N.), urea and NH_3 in rumen ingesta, and also to study the heterogeneous nature of the rumen contents themselves, for it soon became clear that in any experiments carried out *in vivo*, sampling of the rumen contents would present great difficulties. The first paper of this series is therefore concerned with the details of the methods adopted for estimating N.P.N., urea and NH_3, with the heterogeneity of the rumen ingesta of the steer, and with some preliminary *in vivo* experiments.

METHODS

The estimation of N.P.N. in rumen contents

(*a*) *Rumen liquor*. The three protein precipitants which seemed most suitable were trichloroacetic acid, sodium tungstate in H_2SO_4 and alcohol. It is well known that for many substances the estimation of N.P.N. does not result in an absolute value but that different values are obtained by different processes and by slightly different applications of the same process. Tests with rumen liquor were therefore carried out to find how suitable these three precipitants were for the present investigations, and under what conditions they gave reliable values which could be readily duplicated. The sample of liquid rumen contents used contained, after straining through muslin, 2·1% total solids and 80 mg. total N/100 g. The experiments with this sample were planned to study the effect on the N.P.N. value of (1) the concentration of the precipitant, (2) the concentration of the rumen contents in the precipitant, and (3) the time for which precipitation was allowed to continue before filtration.

Precipitation with trichloroacetic acid. Approximately 5 g. of the rumen liquor were accurately weighed into each of a series of flasks and made up to 100 ml. with $CCl_3.COOH$ solution of various concentrations ranging from 2·5 to 20%. The mixtures were thoroughly shaken and then filtered after 24 hr. At the same time further samples of the same rumen liquor were precipitated with 10% $CCl_3.COOH$, but the proportion of the rumen liquor to the total volume of the mixture varied from 1 in 5 to 1 in 50. Again filtration was performed after 24 hr. Finally mixtures were made up consisting of 1 part rumen liquor to 20 parts 10% $CCl_3.COOH$ and portions filtered after 1, 4 and 24 hr. The results are summarized in Table 1.

It appears from these results that with $CCl_3.COOH$ the time which elapses between precipitation and filtration is not of great importance. Up to 4 hr. there was little difference, and after 24 hr. the N.P.N. values had only increased by about 5%. Provided therefore that a definite time be chosen for all determinations, consistent results should be obtained. Decreasing the proportion of rumen contents to $CCl_3.COOH$ solution from 1 in 5 to 1 in 50 increased the N.P.N. value from 24·5 to 28·2, but note that the values obtained for a ratio of 1 in 5 and 1 in 10 differed only very slightly and that the same was true for the two values obtained with ratios of 1 in 20 and 1 in 50. Consistent results should therefore be obtained with this precipitant by keeping the proportion of rumen liquor to $CCl_3.COOH$ approximately constant throughout. The actual concentration of $CCl_3.COOH$ is obviously important, for the higher the concentration the greater is the N.P.N. value. Throughout the present work a 10% solution was used.

Precipitation with sodium tungstate in sulphuric acid. 50 ml. 10% sodium tungstate solution were added to 10 g. of rumen liquor and the mixture made up to 500 ml. with H_2SO_4 of various strengths. It was filtered after 24 hr. The effect of varying the proportion of rumen liquor to precipitant and of varying the interval before filtration was also studied.

Table 1. *Estimation of N.P.N. using trichloroacetic acid as precipitating agent*

(mg./100 g. rumen liquor)

Conc. of trichloroacetic acid (%) (1 part rumen liquor to 20 parts total mixture; filtered after 24 hr.)	N.P.N.	Ratio of rumen liquor to total mixture; made up with 10% trichloroacetic acid (filtered after 24 hr.)	N.P.N.	Time of filtration (hr. after precipitation), 1 part of rumen liquor in 20 parts of total mixture; made up with 10% trichloroacetic acid	N.P.N.
2·5	22·1	1 in 5	24·5	1	25·2
5·0	23·9	1 in 10	25·2	4	25·0
7·0	25·3	1 in 20	27·3	24	26·6
10·0	27·2	1 in 50	28·2		
15·0	33·3				
20·0	35·7				

The results (Table 2) show that when filtration was carried out 24 hr. after precipitation, all concentrations of H_2SO_4 between N and $N/10$ tended to give very similar results, whereas with more dilute acid the values were much higher. These high values were probably due to incomplete precipitation, for it was difficult to obtain a clear filtrate at the greater dilutions. The same explanation probably holds for the higher values obtained with $N/6$ acid when filtration occurred 1 or 4 hr. after precipitation. Table 2 also shows that the ratio of rumen liquor to total volume of mixture has little effect on the N.P.N. value provided 24 hr. elapse before filtration.

Precipitation with alcohol. Similar tests were made using alcohol as the precipitant. The results (Table 3) show that the value for N.P.N. increases as the concentration of alcohol diminishes, due undoubtedly to less complete precipitation as the water content of the alcohol increased, since with 96% alcohol a clear filtrate could be obtained almost immediately, whereas with the 60 and 40% alcohols filtration was slow and tended to give turbid filtrates. With 96% alcohol the N.P.N. values were little affected either by the ratio of rumen liquor to alcohol or by the time between precipitation and filtration.

Table 2. *Estimation of N.P.N. using sodium tungstate and H_2SO_4 as precipitating agent*

(mg./100 g. rumen liquor)

Normality of H_2SO_4 (1 part rumen liquor made up to 50 parts total mixture; filtered after 24 hr.)	N.P.N.	Ratio of rumen liquor to total mixture made up with $N/6$ H_2SO_4	N.P.N. filtered after 1 hr.	4 hr.	24 hr.
N	18·8	1 in 5	21·2	20·9	20·5
$N/2$	18·0	1 in 10	21·5	22·2	19·6
$N/6$	17·6	1 in 20	23·6	24·2	18·5
$N/10$	18·2	1 in 50	27·4	27·6	18·0
$N/12$	24·1	1 in 100	33·6	33·1	19·0
$N/16$	24·0				
$N/20$	26·4				

Table 3. *Estimation of N.P.N. using alcohol as a precipitating agent*

(mg./100 g. rumen liquor)

Conc. of alcohol (% by vol.) 1 part of rumen liquor to 20 parts total mixture; filtered after 24 hr.	N.P.N.	Ratio of rumen liquor to total mixture made up with 96% alcohol	N.P.N. filtered after 1 hr.	4 hr.	24 hr.
96	18·3	1 in 5	18·5	18·5	19·2
80	19·5	1 in 10	17·7	18·7	18·3
60	33·1	1 in 20	16·3	16·3	17·7
40	40·3	1 in 50	16·4	17·7	17·6

It is obvious from these first three tables that when filtration was carried out after 24 hr. the 96% alcohol and sodium tungstate methods gave similar results (17·6–19·2 and 18·0–20·5 mg./100 g. respectively), whereas 10% $CCl_3.COOH$ (1 part rumen liquor to 20 parts total mixture) gave much higher values (26·6–27·3 mg./100 g.) although the same sample of rumen liquor was used for all the results recorded in Tables 1–3. Differences of this order (7–10 mg.) between values found by the alcohol and the tungstate methods and the value by $CCl_3.COOH$ have always been observed when the three precipitants have been compared.

Of the three methods investigated the alcohol procedure is the simplest, but it was observed that if phosphate were added to rumen contents as a buffer when urea or NH_3 was present, the alcohol was liable to precipitate a portion of the NH_3, probably as magnesium ammonium phosphate. The alcohol procedure was therefore of very doubtful value as a routine method. $CCl_3.COOH$ was the next choice in order of simplicity. This substance, however, is expensive and large quantities are not readily obtained in war time. Moreover, owing to the inactivation of urease by $CCl_3.COOH$ the estimation of urea in such a filtrate presents difficulties. It was therefore decided to use sodium tungstate and H_2SO_4 as a general routine procedure, confirming the results periodically by parallel tests with $CCl_3.COOH$.

The method finally adopted was as follows. Approximately 10 ml. rumen liquor, accurately weighed, were added to 50 ml. $N/6$ H_2SO_4 in a 100 ml. flask and the mixture transferred to a 500 ml. volumetric flask with $N/6$ H_2SO_4; 50 ml. 10% sodium tungstate were added and the volume was made up to the mark with $N/6$ H_2SO_4. After standing for at least 24 hr. the mixture was filtered and aliquots of the filtrate were taken for analysis. Various trial experiments showed that the rumen liquor could remain in the 100 ml. flask with H_2SO_4 for at least 2 days before it was made up to 500 ml.; after that, any time between 24 and 48 hr. could elapse before filtration took place without any measurable change in the analytical results.

(*b*) *Solid rumen ingesta.* A sample of the solid rumen ingesta was taken from the rumen at the same time as the sample of the rumen liquor used in the experiments just described. It contained 14·3% of total solids and 290 mg. total N/100 g. The N.P.N. value estimated by 10% $CCl_3.COOH$ was 40·2 mg./100 g., while the corresponding value by the sodium tungstate method was 19·6 and by the 96% alcohol method 20·3. With the solid ingesta therefore, as with the liquor, the alcohol and tungstate methods gave similar results, whereas the figure obtained by using $CCl_3.COOH$ was much higher. Moreover, with the alcohol and tungstate methods the N.P.N. content of the solid ingesta was almost the same as that of the liquid (19–20 mg./100 g.), whereas by the $CCl_3.COOH$ procedure the two values were very different, 40·2 mg./100 g. for the solid and 27·2 for the liquid. The solid and liquid ingesta might be expected to have similar N.P.N. values when estimated on the wet weight, since the solid material, which contains some 86% water, is constantly coming into contact with the liquid and so is likely to be impregnated with it. For this reason, and from these results, the authors believed that in routine work with mixtures of liquid and solid ingesta more accurate and consistent values for N.P.N. would be obtained by the tungstate method than by the use of $CCl_3.COOH$. The reason for the higher values obtained with $CCl_3.COOH$ is not yet known.

The estimation of ammonia. Aliquots of the filtrate from the tungstate precipitation were neutralized with N NaOH using phenol red as indicator. 2 ml. N NaOH were then added in excess and the mixture was distilled for 10–15 min. in a current of steam, the distillate being collected in $N/50$ acid. This procedure gave results identical with those obtained when the acid aliquot was rendered alkaline by addition of solid MgO.

The estimation of urea. Aliquots of the tungstate filtrate were neutralized as in the estimation of NH_3, 10 ml. 0·6% KH_2PO_4 were added as a buffer, followed by 5 ml. of a 30% alcohol urease extract [see Peters & Van Slyke, 1932]. The mixture was incubated at 40–45° for 20 min. 2 ml. N NaOH were then added and the mixture was distilled in a current of steam for 10–15 min. The distillate was collected in $N/50$ acid.

In both the NH_3 and urea estimations blank determinations were made. The accuracy of the method was tested by determining urea in liquid rumen contents to which urea had been added in varying amounts. The results recorded in Table 4

Table 4. *Estimation of urea using tungstate as precipitating agent*

(mg. N/100 g.)

Urea-N added	1·52	3·04	6·08	7·60
Urea-N found	1·44	2·93	6·13	7·54
Recovery (%)	95	96	101	99
Urea-N added	15·2	30·4	60·8	152·0
Urea-N found	15·7	30·2	60·2	151·0
Recovery (%)	103	99	99	99

show that with less than 6 mg. urea-N/100 g. rumen contents the accuracy is about 95%, but that above that value the accuracy closely approaches 100%.

The heterogeneous nature of the rumen ingesta

The bovine rumen is an enlargement of the oesophagus and may contain as much as 200 lb. of material. The upper portions of the ingesta have a consistency similar to that of horse faeces, but as the rumen is descended the mass becomes increasingly moist, until 12–18 in. below the fistula the solid contents are bathed in fluid. At frequent intervals the whole mass of liquid and solid is mixed together by movements of the rumen musculatory system. A sample truly representative of this heterogeneous mass at any given time is difficult to obtain. Determinations of N distribution values on samples taken at the same time from different positions in the rumen showed a variation between extremes of almost 20%, and marked differences were also observed when samples of the material were taken from different depths in the rumen. In Table 5, the total N and N.P.N. values are recorded for moist but solid samples taken from different levels of the rumen. The N.P.N. values in particular show very wide variations. At depths below 8–12 in. the sampling difficulties become greater, for at these levels the proportion of liquid to solid withdrawn can be varied at will by the sampler. The sampling difficulties apparent in the results recorded in Table 5 are therefore much greater for samples taken from lower levels in the rumen where (below 8 in.) the greater part of the rumen contents is to be found.

Table 5. *Concentration of total nitrogen and* N.P.N. *of rumen ingesta from different depths of the rumen*

Depth below surface of ingesta in.	Water %	Fresh rumen contents (mg./100 g.)		Dry matter (mg./100 g.)	
		Total N	N.P.N.	Total N	N.P.N.
1	82·5	342	15·8	1949	90
4	84·3	321	20·1	2044	128
6	85·1	307	11·9	2053	80
8	83·9	288	12·3	1788	77
% difference between extremes	3·1	15·8	40·8	12·9	39·8

One of the aims of these experiments was to feed urea in a typical basal diet and study the changes in the rumen ingesta at intervals after the meal. Preliminary tests were therefore made to detect any variations in the total N of the rumen ingesta throughout the 10 hr. following a urea-free meal. Each sample analysed was a mixture of samples taken from four different parts of the rumen (see Table 6). It will be seen that total N values varied

Table 6. *Nitrogen content of rumen ingesta at hourly intervals after meal*

Time after meal hr.	Moisture %	Total N (mg./100 g.) Fresh rumen contents	Dry matter
1	80·0	360	1800
2	80·2	322	1628
3	80·5	362	1856
4	82·4	302	1716
5	82·0	311	1728
6	81·9	316	1746
7	81·6	322	1750
8	81·7	360	1967
9	81·9	328	1817
10	82·0	312	1733

very irregularly from 311 to 362 mg./100 g. for wet rumen contents, and from 1628 to 1967 mg./100 g. for dry matter. The authors believe that these variations were due mainly to sampling difficulties which made it impossible to detect with certainty the conversion of urea to protein in the rumen after a urea meal unless the amount of conversion were much greater than could be expected.

It was originally intended that extensive *in vivo* experiments should be done. The N distribution in the rumen on a diet containing no added urea was to have been determined. Urea was then to be included and its conversion into protein closely observed. After preliminary experiments, however, it appeared that sampling difficulties made precise

information as to the N distribution throughout the whole of the rumen ingesta at any given time unobtainable. Without such information any attempt to follow the conversion of urea to protein in the intact rumen was of little value.

Another difficulty also arises in interpreting the results obtained in *in vivo* experiments, because portions of the rumen contents are periodically passing from the rumen and reticulum into the other stomachs, and the material passing on at any time may not be representative of the rumen contents as a whole. It follows that if the small amount of urea or ammonia N present in the rumen immediately after a urea meal decreased within the next few hours, the decrease might be partly due to the passing of water-soluble urea or NH_3 into the other stomachs and intestines, and not necessarily to their conversion to protein. Even a simultaneous increase in the amount of total N in the dry matter of the rumen contents might be due to a selective passing-on of water-soluble carbohydrates and other constituents of the diet. These difficulties are illustrated by the results of an experiment in the *in vivo* section of the general investigations.

For 6 months the steer had received daily a mixture consisting of 2¾ lb. oats, 2 lb. bran, 1 lb. starch, ⅜ lb. molasses, 40 g. urea, 14 lb. hay and water *ad lib.* During this particular experiment the concentrate mixture was given in two portions, one at 9.45 a.m., the other at 4.30 p.m. Samples of rumen contents were removed at 9.30 a.m., 10.30 a.m., 12.30 p.m. and 3.30 p.m. The 9.30 samples should therefore have been representative of the rumen ingesta after a fast of 17 hr., the others of the material at varying periods immediately after the meal. At each sampling period semi-solid samples of approximately 600 g. were collected from near the top at each of the four 'corners' of the rumen and a fifth more liquid sample from deep down in the centre of the rumen. Without further treatment these five samples were analysed immediately in duplicate for water, total N and N.P.N. contents. Three days and also 6 days later this procedure was repeated. Thus, while the steer was receiving the basic diet plus urea, 96 values were obtained for the content of both the total N and the N.P.N. in the semi-solid ingesta, and 24 values for both these constituents in the liquid ingesta. Urea was then omitted from the basic diet and 9, 12 and 15 days later, the sampling and analytical procedure were repeated. The detailed results are too numerous to publish in their entirety. It appeared, however, that with both the semi-solid and the more liquid samples duplicate determinations for the same sample frequently differed by 20% or more. A similar difference was often observed in the values obtained for samples taken at the same time but from different parts of the rumen. Such large divergencies in individual values were too frequent to permit conclusions to be drawn as to the synthesis of protein from urea in the rumen.

The average results (Table 7) gave no evidence either for or against the theory that urea is utilized in this way. A study of Table 7 shows the following points:

(1) The concentrations of crude protein, true protein and N.P.N. were higher in the dry matter of the liquid ingesta than in that of the semi-solid material. In any quantitative experiment therefore the proportion of solid to liquid ingesta, and in fact the total amounts of each in the rumen, would have to be known.

(2) There was no significant difference between the protein contents of the rumen ingesta when the diet was fed alone and when it included urea.

(3) The only significant changes on the urea diet were in the N.P.N. fraction, e.g. 144 mg./100 g. in the dry matter of the liquid ingesta just before a meal had increased to 313 mg./100 g. 30 min. after the meal and had fallen again to 119 mg./100 g. some 5½ hr. later; the corresponding values for the semi-solid ingesta were 74, 118 and 59 mg. N/100 g.

Table 7. *Three-day average values for the content of crude protein, true protein and N.P.N. in the rumen ingesta on a diet containing urea and on the same diet without urea*

Time after previous meal hr.	Crude protein % dry matter		True protein % dry matter		N.P.N. mg. N/100 g. dry matter	
	Urea	No urea	Urea	No urea	Urea	No urea
	Semi-solid ingesta					
17	11·6	10·6	11·1	10·2	74	62
0·5	10·1	10·3	9·4	9·9	118	68
3	10·2	10·1	9·7	9·8	83	44
6	11·0	10·7	10·6	10·3	59	58
	Liquid ingesta					
17	15·1	17·2	14·2	16·7	144	83
0·5	17·3	15·6	15·3	14·8	313	130
3	16·3	14·9	14·6	14·1	276	128
6	15·3	15·8	14·6	14·9	119	139
Average of 96 duplicate determinations involving 48 samples of semi-solid ingesta	10·7	10·4	10·2	10·0	84	58
Average of 24 determinations involving 12 samples of liquid ingesta	16·0	15·9	14·7	15·1	213	120

Crude protein = 6·25 × total N; true protein = 6·25 × (total N − N.P.N.).

To show that urea was converted to protein in the rumen such an experiment should yield results such that the decrease in N.P.N. following the urea meal could be correlated with an increase in the 'true protein'. But here, if the fall in N.P.N. were entirely due to protein synthesis, a decrease in N.P.N. from 118 to 59 mg. N/100 g. and from 313 to 119 would mean an increase in true protein of only 0·4 and 1·2 % respectively. The value of 9·3 % for the true protein of the semi-solid ingesta 30 min. after the meal (Table 7) would therefore have to increase by 0·4–9·7 %, some hours later, an increase which would not be significant owing to sampling errors. Similarly, the value of 15·3 % for the liquid ingesta would have to increase to 16·7 %. Actually in the solid material the value rose to 9·7 % after 3 hr. and 10·6 % after 6 hr., but in the liquid ingesta it decreased to 14·5 %. These results therefore supply no proof of protein synthesis from urea. They are, however, not at variance with the suggestion that protein synthesis from urea did take place, because throughout the day the animal continued to drink at intervals and to secrete the usual large quantities of saliva. Moreover, part of the ingesta, particularly in the more liquid form, would be continually passing from the rumen. These processes would all tend to induce a diminution in protein and in N.P.N. content of the liquid ingesta as the interval after the meal increased. Simultaneously the semi-solid rumen ingesta might become slightly richer in protein because the more soluble carbohydrate material was washed out of the animal's food. By increasing the urea in the diet during the urea period the problem would not be solved but made more difficult, as the greater urea consumption would increase the water ingested and this would enhance the protein content of the solid ingesta and decrease that of the liquid.

DISCUSSION

The detection of urea conversion to protein by an *in vivo* experiment of this type is extremely difficult and seems never likely to yield results completely beyond dispute. Thus it seemed wise, at least in the early stages of the work, to concentrate on incubation experiments carried out *in vitro* when these difficulties would not arise, and to postpone the *in vivo* section of the general investigations for the time being. The main portion of the work completed therefore consists almost entirely of the *in vitro* experiments to be discussed in the next two papers of this series.

Since the original *in vivo* programme was temporarily abandoned, Wegner, Booth, Bohstedt & Hart [1941] have published a description of *in vivo* experiments using a heifer with a rumen fistula. The values for N.P.N. and total N of the rumen contents at intervals after the ingestion of a basal diet were compared with the corresponding values obtained when the diet included urea. Wegner *et al.* found that in general the crude protein content of the rumen ingesta was higher with added urea than it was on the basal diet alone, and that the N.P.N. of the ingesta fell to the basal level during the first few hours after a meal. They therefore suggested that urea utilization takes place in the rumen and that it occurs within 4–6 hr. after ingestion. An examination of the results, however, shows that such an interpretation is not necessarily correct, and that these workers have evidently encountered those very difficulties which the present authors anticipated. Thus from their 1st experiment it can be calculated that on the basal diet alone the true protein of the rumen ingesta appeared to increase from 8·4 % at 1 hr. after a meal to 11·3 % 4 hr. later, a difference of 2·9 %. This was accompanied by a decrease in N.P.N. equivalent to only 1·0 % true protein. When, however, the diet included urea, the corresponding increase in true protein was only 0·9 % (9·9–10·8 %), and this was accompanied by a decrease in N.P.N. equivalent to 1·5 % protein. There therefore appears to have been less synthesis of protein with urea than with the basal diet alone during the 5 hr. following the meal. Again, the value of 11·3 % for the true protein 5 hr. after the basal diet was fed, was actually slightly higher than 10·8 %, the corresponding value when urea was included in the diet, a condition reversed 4 hr. later without any significant change in N.P.N.

In their 2nd experiment Wegner *et al.* [1941] fed a very abnormal basal diet consisting of 15 lb. corn silage and 3 lb. starch, following this by a period on the same basal diet with the addition of urea amounting to 5 % of the dry matter of the ration. They found that of several diets which they tested, this most unusual one, which must have contained only about 5 lb. dry matter, gave the most marked results. Their values show that on two of the days when the basal ration was fed alone, the crude protein content of the rumen ingesta increased by 0·45 and 0·85 % between either 1 or 2 and 8 hr. after the meal, but that on the only other day for which the corresponding values are available it decreased by 1·7 %. No conclusion can be reached therefore as to whether the crude protein content of the rumen ingesta should be expected to rise or fall during the first 8 hr. after the ingestion of the basal diet. On the 2 days for which the corresponding values are given for the basal diet + urea, the crude protein decreased by 0·7 % between 2 and 8 hr. after feeding on the first day, and remained unchanged on the second. Even correcting the crude protein for NH_3 where possible (N.P.N. values are not given), the maximum increase in protein following the ingestion of urea was only 0·6 %. In relation to the changes occurring on the

basal diet alone, the results do not support the view that urea is converted to protein in the rumen during the first 8 hr. following a meal.

The evidence upon which Wegner *et al.* [1941] appear to place most confidence is that on the basal diet alone the crude protein content of the rumen ingesta varied from 8·9 to 10·6 %, whereas with the addition of urea to the diet this value became much higher and varied between 12·0 and 12·7 %. In all probability, however, these values might be due to the fact that when 100 g. urea were added to the diet which supplied only 5 or 6 lb. dry matter per day, of which 3 lb. was starch, water consumption and also salivation would be much increased. This would cause the starch and the more soluble matter in the corn silage to be washed from the rumen much more rapidly than in the absence of urea. The proportion, in the dry matter of the rumen ingesta, of fibre and of the less soluble protein enmeshed in it would thus be increased.

These experiments of Wegner *et al.* [1941] therefore confirm the views held by the present authors that *in vivo* experiments of this type cannot be expected to yield any certain evidence of protein synthesis from urea until truly representative samples of the total ingesta can be obtained and analysed, and until more is known of the effect of urea on the passage of the various dietary constituents through the rumen.

SUMMARY

1. The relative merits of trichloroacetic acid, sodium tungstate with H_2SO_4, and alcohol have been compared as precipitants in the estimation of N.P.N. in rumen ingesta.

2. A description is given of the methods finally adopted for the estimation of N.P.N., urea and NH_3.

3. The heterogeneous nature of the rumen contents is described and illustrated by analyses.

4. The difficulties involved in the interpretation of the results obtained by *in vivo* experiments are discussed.

5. Owing to these difficulties, the results of *in vivo* experiments of this type cannot be regarded as supplying evidence either for or against the theory that urea is converted to protein in the rumen.

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The Utilization of Urea in the Bovine Rumen. 2. The Conversion of Urea to Ammonia

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In the previous paper of this series [Pearson & Smith, 1943] evidence was advanced to show that the interpretation of experiments carried out *in vivo* on the utilization of N.P.N. by ruminants was so difficult as to render the work of little value and that more reliable results could probably be obtained by *in vitro* methods. A plan of investigation was therefore prepared in which samples of rumen ingesta were to be incubated in the laboratory with and without urea, and the N partition studied at intervals throughout the incubation period. At the outset of the work it appeared that even when 40 g. urea were fed per day to a steer with a rumen fistula, no urea could be detected in the rumen ingesta. In preliminary incubation experiments it was also found that 100 g. rumen contents were able to convert some 100 mg. urea to NH_3 in 1 hr. It is probable therefore that any utilization of urea by ruminants involves the conversion of urea to NH_3 as the first stage of the process. A detailed study of this conversion has therefore been made, with particular reference to the effect upon it of various factors such as temperature, pH, concentration of urea, nature of the gases present during incubation and certain inhibitory substances. The present paper consists of a brief description of these experiments.