For instance, the iodophile populations of the rumen of sheep and oxen alike show a marked increase in density in a 6 hour incubation period. The predominant micro-organisms from the ox are, however, bacteria which synthesize starch (Baker, 1942), while, in the sheep, they are pseudo-yeasts and other organisms which synthesize glycogen (Quin, 1943). Among the yeasts is the organism referred to by Quin as *Schizosaccharomyces ovis*. It seems reasonable to state, therefore, that diet may induce marked quantitative changes in the characteristics of the bacterial population but that the general qualitative characteristics persist.

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The Formation of Protein

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Introduction

During the last 40 years the claim has frequently been made that for some animals a portion of the dietary protein required to maintain normal health and growth can be replaced by substances containing only non-protein nitrogen (N.P.N.). The work supporting or refuting the claim has been reviewed fairly recently by Krebs (1937) and by Owen (1941). In discussing the earlier researches carried out mainly on the continent Krebs cites 126 references. He shows that the earlier results and conclusions were conflicting and that the conflict of opinion arose mainly from the fact that the earlier experiments were often so badly planned and so inadequately controlled that the findings did not lend themselves to decisive interpretation.

Until the manufacture of great quantities of non-protein nitrogenous compounds such as urea from atmospheric nitrogen became possible, and until it appeared that war might again curtail the supplies of available feeding stuffs, the question was regarded as of little importance in Britain. During the past few years, however, the subject has been studied in much detail both here and in America with the result that the problem which was hitherto so confused has now been greatly clarified.

The object of this paper is to summarize the recent work. The results of the investigations fall naturally into 2 sections, evidence obtained for the conversion of N.P.N. to protein from practical feeding trials, and evidence which supports the principal theory put forward to explain the ability of the ruminant to utilize N.P.N. in this way.

Feeding Trials

Milk Production

At the Hannah Institute, the value of urea feeding for milk production has been investigated by Owen, Smith and Wright (1943). Seven cows were kept in specially constructed metabolism stalls in which their urine and faeces could be collected separately and weighed. In the main experiment 5 animals were first given a balanced diet in which 25 per cent. of the total nitrogen intake was supplied as blood meal, an excellent source of protein for milk production. After 3 or 4 weeks on this diet, the blood meal was replaced by its nitrogen equivalent of urea, and the urea mixture was given for 5 or 6 weeks, and then removed from the diet. The urea diet was readily eaten and no toxic effects were observed. The composition of the milk was not adversely affected by ingestion of urea and the weights of the animals were as well maintained as on the blood meal diet. There was no significant decrease in milk yield when urea replaced blood meal but, on withdrawing urea from the diet, the yield immediately fell. Nitrogen determinations in urine and faeces showed that urea feeding led to a slightly increased nitrogen excretion. This suggests, as might well be expected, that a portion of the dietary urea passes through the rumen and reaches the blood stream without being converted to protein. Some of this will return to the rumen in the saliva, but part of it will undoubtedly be excreted in the urine. The amount of urea which was found to be excreted unused averaged 25 per cent. of the total ingested. The remaining 75 per cent. appeared to be efficiently utilized. In practice much of the unused 25 per cent. would not be wasted for it would ultimately reach the land in the dung.

In experiments at Wisconsin by Rupel, Bohstedt and Hart (1943), 25 Holstein cows were used to compare a basal ration poor in protein with the same ration supplemented with urea or linseed meal in such a way that the supplements supplied about 44 per cent. of the total nitrogen. The experiment was continued for 3 complete lactations. A study of many factors such as the yield and composition of the milk, the live weights of the cows, and the weights of the calves born to the cows, showed that with a ration containing sufficient carbohydrate, urea can function as a source of nitrogen as effectively as linseed meal. Rupel and his colleagues recommend that, for conditions typical of Wisconsin, where hay, silage and home grown grains are given, and the grains are rich in carbohydrate and relatively poor in protein, a suitable nitrogen level in the concentrate mixture can be secured by adding 3 lb. urea to 97 lb. grain mixture.

Growth

Three recent experiments will suffice to illustrate the conversion of N.P.N. to protein by young growing stock.

In experiments by Bartlett and Cotton (1938), heifers between 7 and 17 months old were divided into groups of which one received a basal ration low in protein, another the basal ration supplemented with urea, and a third the basal ration brought up to a normal protein standard by addition of decorticated groundnut cake. In the urea ration, urea replaced about 30 per cent. of the total nitrogen of the normal protein ration. The average daily gain in weight over a period

of 142 days was 0·99 lb. on the low protein diet, 1·23 lb. on the urea diet and 1·39 lb. on the normal protein diet. The difference between the increases on the urea and normal protein diets was not statistically significant, but both these increases were significantly greater than the increase on the low protein diet. There were no significant differences in skin handling properties, fatness and body measurements.

In the main experiment by Hart, Bohstedt, Deobald and Wegner (1939), 6 heifer calves were used. Calf 1 received a basal diet which was just sufficient for maintenance but not for growth. Calves 2, 3 and 4 received the basal diet supplemented with sufficient urea to supply 43, 61 and 70 per cent. of the total nitrogen. Calf 5 received the basal diet with sufficient ammonium bicarbonate to supply 69 per cent. of the total nitrogen. Calf 6 was given the basal diet with enough casein to supply 66 per cent. of the total nitrogen. The following are the most important results:

(1) During 12 weeks on the basal diet alone, Calf 1 gained no weight. Urea was then added to its diet so that it was receiving the same food as Calf 2. It then put on an average of 1 lb. daily for a period of 36 weeks. This gain in weight was brought about solely by the addition of urea to the basal diet.

(2) The growth rates of Calf 2 receiving 43 per cent. nitrogen as urea and Calf 6 receiving 66 per cent. nitrogen as casein were very similar. Both gained about 0·9 lb. daily over a period of some 24 weeks.

(3) Calculated from the growth curves given by the authors, the growth rates of Calves 3, 4 and 5 were lower than those of Calves 2 and 6, the average increase in weight being about 0·5 to 0·6 lb. daily. Feeds in which urea and ammonium bicarbonate supplied as much as 61 and 70 per cent. of the total nitrogen were, therefore, much inferior to feeds in which urea supplied only 43 per cent. At the highest level of urea feeding with Calf 4, marked diuresis occurred and on *post mortem* examination evidence of kidney damage was detected. No diuresis or kidney damage was observed with urea at the 43 per cent. level. It appears, therefore, that for the best results urea should not supply more than about 40 per cent. of the total nitrogen of the diet.

(4) Detailed analysis and *post mortem* examination showed that the tissues and the protein of the tissues were normal in the animals which grew so well when receiving urea.

In experiments by Millar (1944), the source of N.P.N. was ammoniated sugar beet pulp which contains 4 or 5 per cent. of readily available nitrogen. A process for the manufacture of this product has been described and patented (Millar, 1941, 1942). Several male calves were used in the feeding trials. Two calves received first the basal ration containing only 7 per cent. protein. After 150 days, during which they increased in weight by less than half a pound daily, the ordinary beet pulp of the diet was replaced by ammoniated beet pulp, bringing the percentage of crude protein (N × 6·25) up to 17. This change brought about an increase in weight of about 2 lb. daily during the next 10 weeks. A third calf received at first sufficient ammoniated beet pulp to raise the percentage of crude protein of the

diet to 17. At 132 days, the proportion of ammoniated beet pulp was reduced so that the total crude protein in the diet amounted only to 12·4 per cent. This particular calf grew more rapidly on the diet in which the crude protein content was less and in which N.P.N. supplied only 44 per cent. of the total nitrogen than it did on the diet in which the proportion of N.P.N. was raised to 60 per cent. A fourth calf received the basal diet except that ordinary beet pulp was replaced by ammoniated beet pulp so that the percentage of crude protein was raised from 7 to 12·4. It gained 1·62 lb. daily as compared with 1·96 lb. for a fifth calf which received the basal diet supplemented with soya bean meal so that the true protein content of the diet was raised from 7 to 17 per cent. The ammoniated beet pulp, therefore, gave a gain in weight equivalent to just over 80 per cent. of that given by soya bean protein.

Millar found that no excessive diuresis occurred with any of the animals nor was there any evidence of kidney trouble. The protein content of the grown animals, and the colour and flavour of the meat were normal. It is evident, therefore, that while ammoniated beet pulp may be slightly inferior to soya bean as a source of protein for growing calves, it is certainly capable of replacing a very substantial proportion of the protein in the diet.

Wool and Meat Production in Sheep

Experiments by Nehring and Schramm (1939), Kirsch and Sauer (1938), and Mangold and Stotz (1937) have all shown that urea can replace a proportion of the normal protein content in the diet of sheep for both growth and wool production. More recently, the utilization of urea by sheep for maintenance and growth has been ably confirmed by Harris and Mitchell (1941, 1, 2) and by Johnson, Hamilton, Mitchell and Robinson (1942).

Maintenance of Non-Ruminants

N.P.N. has been shown to be of no value in the feeding of rats (Kriss and Marcy, 1940) and pigs (Schmidt-Ewig, 1928).

Conclusions from These and Other Feeding Trials

1. In favourable conditions urea can be utilized by ruminants for the production of meat, milk and wool.

2. The proportion of nitrogen supplied as urea should not exceed 40 per cent. Probably 25 per cent. would be a more efficient level to adopt.

3. Sufficient readily available carbohydrate must be present in the diet to balance all the nitrogen which it contains. From this it follows that urea may prove to be of particular value in areas where the grain is specially rich in carbohydrate and relatively poor in protein.

Recent Evidence which Supports the Theory Usually Advanced to Explain the Utilization of Non-Protein Nitrogen by Ruminants

The principal theory for the utilization of N.P.N. is that the bacteria which abound in the rumen utilize it as a source of nitrogen for their own growth and multiplication, and that in doing so they convert it to protein. When eventually this bacterial protein passes from the rumen into the

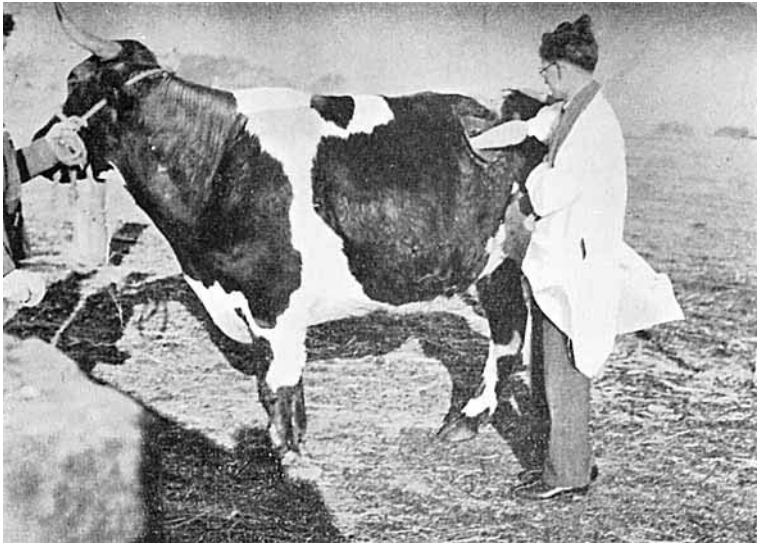


FIGURE 1. THE REMOVAL OF RUMEN CONTENTS FROM THE FISTULA.

remainder of the digestive tract, it is assumed to be digested with the other digestible components of the diet.

In order to collect evidence for or against this theory, experiments were begun a few years ago at the Hannah Institute (Pearson and Smith, 1943) where there was an animal with a rumen fistula (Figure 1) from which large volumes of rumen ingesta could readily be removed. The object of the work was to incubate rumen contents with urea and determine whether formation of protein could be detected, the conditions existing in the intact rumen being imitated as far as possible in the laboratory. To overcome sampling difficulties, the more liquid contents, probably constituting about 80 per cent. of the total rumen ingesta, were used throughout the work.

Results of a typical incubation experiment can be outlined briefly as follows. The total nitrogen remained constant, the urea was very rapidly converted to ammonia, and the N.P.N. decreased. The decrease in N.P.N. took place mainly if not entirely in the ammonia fraction. By subtracting the values for N.P.N. from those for total nitrogen, it appeared that protein was synthesized relatively rapidly during the first 3 hours of incubation.

During the course of this work at the Hannah Institute, Mr. Frank Baker of Guildford has given most valuable help on the microbiological side in which he has had so much experience. The methods he has adopted for counting the bacteria and for reporting on the samples are described elsewhere (Smith and Baker, 1944). By making a thorough examination of the many formolized samples sent to him from the Hannah Institute, Baker (1943) was able to show that the apparent increase in protein during the first 3 hours of incubation occurred while the microbiological conditions in the rumen liquid bore a very close resemblance to those of the initial sample as it came from the rumen. After incubation for about 6 hours, however, protein formation was no longer detectable, autolysis set in and the microbiological conditions in the incubated liquid began to differ markedly from those of normal rumen contents. The fact that protein synthesis occurred only in the first few hours of incubation makes it reasonable to assume that protein synthesis readily occurs in the intact rumen provided the conditions are favourable.

The amount of N.P.N. converted to protein during incubation *in vitro* was frequently of the order of 8 mg: N per 100 g. rumen liquid. This appears at first sight to be small but, if protein synthesis were to proceed at the same rate in the intact rumen, 300 g. protein would be synthesized in one day. This is roughly a third of the protein requirements of a cow yielding 2 or 3 gallons of milk daily.

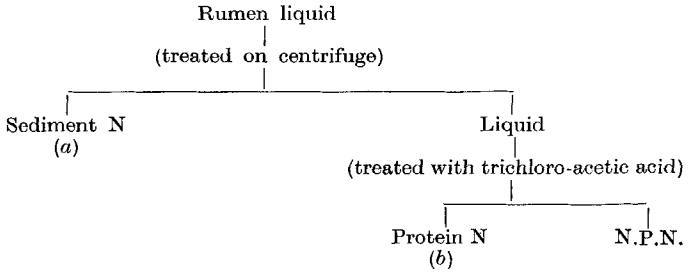
These results indicate that N.P.N. is almost certainly converted to protein during incubation, but at this stage of the work at least 3 questions still required to be answered: 1. Is the change in N.P.N. brought about by biological processes or is it solely a chemical change? 2. If it is caused by micro-organisms, to which particular micro-organisms should it be attributed? 3. Can the protein be isolated in a form in which it will be possible to estimate its biological value and digestibility? In an endeavour to answer these 3 question, the following experiments were carried out.

1. *Incubation with Sodium Fluoride.* A sample of rumen contents was divided into a number of portions each of which was incubated with a different concentration of NaF, and the synthesis of protein after 2 hours was estimated. It was found that, as the concentration of NaF increased from 0 to 0·03 per cent., protein synthesis was inhibited and at 0·3 per cent. marked hydrolysis of protein occurred. With 1 per cent. NaF no change occurred.

2. *Incubation at Different Temperatures.* Portions of another sample of rumen liquid were incubated at a series of different temperatures from 0° to 90° C. Synthesis was found to reach a maximum between 30° and 40° C. Above 40° C. hydrolysis of protein predominated. At 80° or 90° C. little or no change was observed.

From these experiments 2 conclusions follow. The changes observed during incubation are almost certainly of microbial origin. Both synthesis and hydrolysis of protein probably proceed simultaneously in the rumen, the one or the other predominating according to the conditions in the rumen at the time.

3. *Isolation of the Synthesized Protein and Correlation between Synthesis of Protein and Bacterial Activities.* By centrifuging the rumen liquid after incubation for about 1 hour at 3000 r.p.m. a sediment was obtained which contained the synthesized protein. The following scheme was therefore adopted for isolating this sediment and for estimating the amount of true protein synthesized.



Total protein N = (*a*) + (*b*)

Table 1 shows the results of a typical experiment in which this fractionation scheme was used. When carbohydrate was present, the weight of sediment and the total protein increased while the N.P.N. decreased. There was also a very marked increase in the numbers of iodophile bacteria. In the absence of added carbohydrate, protein hydrolysis slightly predominated. The total protein N and the bacterial count decreased while the N.P.N. increased. This experiment, therefore, showed that adequate amounts of carbohydrate must be present if N.P.N. is to be converted to protein, and that synthesis is accompanied by an increase in the iodophile bacteria described by Baker (1943).

In another experiment the rumen liquid was centrifuged for different times and at different speeds before it was incubated. Samples were removed from the supernatant liquid for obtaining the initial sediment by further centrifuging and for estimating the initial N.P.N. and other values. The remainder of the supernatant liquid was incubated for

6 hours. The results which are summarized in Table 2 lead to 2 main conclusions.

TABLE 1

SYNTHESIS OF PROTEIN AND INCREASE IN IODOPHILE COUNT ON INCUBATION OF RUMEN CONTENTS IN PRESENCE OF ADDED CARBOHYDRATE

Weights and nitrogen values are expressed in mg. per 100 g. rumen liquid

Value	Sediment		Trichloro-acetic acid precipitate		N.P.N.	Relative macro-iodophile count (Smith and Baker, 1944)
	Weight	N	Weight	N		
	With added carbohydrate					
Initial ..	146	15·0	289	19·1	53·2	2
Final ..	485	33·2	222	14·6	40·1	50
Change ..	+339	+18·2	−67	−4·5	−13·1	+48
	Without added carbohydrate					
Initial ..	146	15·1	234	19·5	52·2	2
Final ..	131	13·9	230	17·7	54·7	0
Change ..	−15	−1·2	−4	−1·8	+2·5	−2

TABLE 2

THE EFFECT ON THE PROTEIN CONTENT AND ON THE COUNTS OF VARIOUS ORGANISMS OF CENTRIFUGING THE RUMEN LIQUID BEFORE INCUBATION

Experiment		A	B	C	D
Centrifuge { speed (r.p.m.)		—	1000	2000	3000
Centrifuge { time (minutes)		—	3	15	25
Initial sediment (mg. per 100 g.)*		1388	127	77	33
Increase during incubation (mg. per 100 g.)		227	231	289	223
Protein synthesis (mg. per 100 g.)		11·3	15·2	15·7	11·3
Relative count of organisms					
Protozoa	initial ..	17	0·6	—	—
	final ..	12	0·0	—	—
Amylospirillum	initial ..	2	2	—	—
	final ..	4	3	—	—
Amylobacterium	initial ..	0·4	0·3	—	—
	final ..	0·9	0·8	—	—
Amylococcus chains ..	initial ..	21	9	2	0·6
	final ..	44	27	13	5·0
Zoogloea (relative volume) ..	initial ..	—	0·1	0·1	0·1
	final ..	—	0·5	0·7	0·5

* Obtained by centrifuging the supernatant liquid for 1 hour at 3000 r.p.m.

First, the preliminary centrifuging in experiments B and C naturally greatly reduced the initial sediment obtained by centrifuging the supernatant liquid for 1 hour at 3000 r.p.m. The amount of synthesis, however, was not diminished but was actually slightly increased. It was, therefore, possible by a preliminary centrifuging process to obtain a liquid which on incubation yielded a sediment in which at least 70 per cent. had actually

been synthesized during the incubation. Thus in experiment C an increase of 289 mg. per 100 g. rumen liquid occurred on an initial value of 77 mg., as compared with experiment A where the increase was only 227 mg. on 1388 mg. Experiments are proceeding at the present time in which it is hoped to isolate a sufficiently large quantity of the type of sediment obtained after incubation in experiments B and C so that its digestibility and biological value may be determined.

Second, the protein synthesis was actually greater in experiments B and C than in experiment A, in spite of the fact that the preliminary centrifuging in experiments B and C had removed the protozoa, many of the larger iodophile bacteria and much of the initial sediment originally present in the rumen liquor. Baker (1943) has described the iodophile bacteria as falling into 2 groups, the macro-iodophiles and the micro-iodophiles. The larger organisms can be readily counted but the smaller ones form masses of zoogloea in which it is impossible to count the individual bacteria. A measure of the volume of zoogloea can, however, be obtained and, from this and several other experiments, it is concluded that the main contributors to the protein synthesis detected by chemical analysis are the micro-iodophile bacteria. The macro-iodophiles though readily counted are not present in sufficient amount to make more than a very small contribution to the total protein synthesis. In growing and multiplying, these iodophile bacteria synthesize not only protein but also a polysaccharide which is indistinguishable from starch. When this polysaccharide was estimated at intervals during incubation the results suggested that in the early part of the incubation the iodophile bacteria built up and stored polysaccharide much more quickly than they multiplied, and synthesized protein. Again after some time, 2 to 3 hours with this particular sample, when multiplication ceased, loss of polysaccharide preceded hydrolysis of protein (Smith and Baker, 1944).

The Nature of the Sediment after Incubation. It is essential that the digestibility and biological value of the sediment after incubation should be known. As already stated, large amounts are being prepared to enable this to be done. In the meantime it is of interest to compare its general chemical composition with that of a typical feeding stuff like linseed cake. The marked similarity between the two is shown in Table 3.

TABLE 3

COMPARISON OF THE COMPOSITION OF THE SEDIMENT OBTAINED AFTER INCUBATION AND OF LINSEED CAKE

Constituent	Sediment per cent.	Linseed cake per cent.
Moisture	0·5	0·5
Ash	6·2	5·8
Crude protein (N × 6·25)	36·3	33·0
Total carbohydrate ..	46·8	50·0
Lipoids	9·5	10·7
Total	99·3	100·0

The values for the sediment are those found for a typical sample after being washed with alcohol and ether. The values for the linseed cake are taken from standard tables and corrected to 0·5 per cent. moisture.

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Summary and Conclusions

1. Feeding trials have shown that urea can be of value in the diet of ruminants for the production of meat, milk and wool, provided the diet is properly balanced.

2. Extensive experiments carried out *in vitro* with liquid ingesta immediately after their removal from the rumen have shown that protein synthesis and hydrolysis can proceed simultaneously and that either may predominate according to the conditions existing at the time.

3. Readily available carbohydrate, starch or simple sugar, must be present in sufficient amount and the diet must be adequately balanced if non-protein nitrogen is to be converted to protein.

4. The greater portion of the synthesis is attributed to the masses of micro-iodophile bacteria which abound in the rumen. The protozoa do not appear to contribute to the actual synthesis.

5. The synthesized material can now be isolated in a form in which its digestibility and biological value can be estimated. In general composition it closely resembles a typical feeding stuff such as linseed cake.

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Discussion

Mr. M. H. M. Arnold (Research Department, Imperial Chemical Industries, Ltd., Billingham, Durham), opener: The percentage transfer of ingested nitrogen to milk in the Hannah experiments of Owen, Smith and Wright (1943) was at least as high with diets containing urea as with those containing blood meal. I calculate that the efficiency was 28·3 and 27·3 per cent. for the urea diet, and 28·6 and 23·4 per cent. for the blood meal diet. In these experiments only part of the total nitrogen was derived from urea or blood meal, and the efficiency of transfer of this excess nitrogen was 14·7 and 12·1 per cent., respectively. The cows were thus given nitrogen at a rather too high level and I should like to see these experiments repeated at a lower level of nitrogen intake.

Similar calculations have been made from the results of other workers, but the information contained in their papers is insufficient for precise estimates. Approximate values indicate that inorganic nitrogen is inferior to ordinary protein in conversion to meat but at least equal to it for conversion to milk. No reason for this difference can be given.

Just as fertilization of land results in a change of the predominant soil flora to types best able to utilize the increased amount of nutrients, so prolonged feeding of urea might change the microflora of the rumen in the direction of greater nitrogen efficiency. Urea is a labile substance and there is risk of loss of ammonia and of denitrification if it is not rapidly absorbed. Striking results might be obtained with urea feeding if it was sufficiently prolonged to allow maximum adaptation of the ruminal flora.

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Mr. R. Benesch (L.C.C. Central Pathological Laboratory, Epsom, Surrey): Dr. Smith and Mr. Arnold have raised the point about the conversion of urea in the rumen into ammonia with the subsequent possibility of the loss of ammonia both directly and indirectly by way of the blood stream and kidneys. Therefore, the form in which the urea is given may be a decisive factor in its utilization. In addition, it is generally acknowledged that an easily available carbohydrate must be present if the inorganic nitrogen is to be used efficiently. The ammonia nitrogen derived from the hydrolysis of the urea should be liberated gradually in order to prevent a sudden explosive effect with consequent toxic effects on the rumen microflora as well as on the animal itself. For all these reasons various patents for preparing urea potato flakes or urea sugar beet slices were taken out by the I.G. in Germany. In all these preparations urea was supposed to be present in an adsorbed condition and was, therefore, assumed to be liberated only gradually on reaching the rumen, with a consequently greater chance for the urea nitrogen to be synthesized into protein nitrogen. There are, however, naturally occurring substances, which, in addition to being carbohydrates, possess an acidic structure and could therefore hold ammonia by chemical forces. I think that ammonium alginate might be an ideal example of such an ammonia-containing carbohydrate. I gave ammonium alginate to hens in 1941 but was unable to find any utilization of inorganic nitrogen. I wonder whether this substance, which I know to be available in this country, could be tried on ruminants.

With regard to the slightly smaller efficiency of protein formed from urea as compared with the usual dietary proteins, it is interesting to hear that the biological value of the synthesized material will be tested. The inferiority of this microbial protein may perhaps be related to the methionine deficiency of "food yeast".

It has been mentioned that the pig and the rat cannot utilize inorganic nitrogen. It was suggested to me by Dr. Harris in 1941 that in refected rats, where vitamin synthesis is known to take place, such utilization may conceivably occur. If this was so, it would be a great help in these investigations in view of the greater convenience with which rats can be dealt with as compared with ruminants.

I would like to know why, in view of the fact that it has now been definitely discovered in this country that urea is of value at least in milk production, nothing has been done about using it on a large scale.

Finally, I want to suggest that the "sterilizing" sulphonamides, which have been found so very useful in elucidating the role of the intestinal flora in other animals, including man, may throw some light on this problem in ruminants as well.

Dr. S. Bartlett (National Institute for Research in Dairying, University of Reading): Members may be interested to hear a brief outline of a series of experiments which have been carried out at the National Institute for Research in Dairying, Shinfield, on feeding cattle with urea. The results of the first experiment carried out in 1937 were published and showed that the live weight increase of young dairy cattle on a low protein ration was improved by the addition of urea but that the improvement was less than that which occurred when extra protein was given (Bartlett and Cotton, 1938).

During the winter of 1939-40 a series of experiments was started. Publication of the results was delayed for security reasons, but these are no longer operative. The first feeding experiment was with nearly 300 milking cows selected from 12 widely separated herds. The cows were divided into groups of 4 and each animal was allocated by lot to one of 4 treatments which involved administration of one of 4 concentrate cubes as a supplement to a basal ration of bulky food. The following cubes were used: (*a*) A cube of low protein content (13 per cent.); (*b*) the same with addition of 2 per cent. urea which raised the value for "protein" (N $\times$ 6·25) to 18 per cent; (*c*) a cube of normal protein content (18 per cent.); (*d*) the same as (*c*) with addition of 2 per cent. urea which raised the value for "protein" (N $\times$ 6·25) to 23 per cent.

As judged by milk yields over a 7 week period, the best results were given by treatment (*c*) followed by (*d*), while treatments (*a*) and (*b*) were about equally poor.

Our interpretation of these results is that while urea is of nutritive value it produces ill effects also. Consequently, with animals on a normal protein diet, the effects of urea are detrimental whereas, with animals on a low protein diet, the ill effects may be outweighed by the benefits.

Further experiments on the herd of the National Institute for Research in Dairying brought out the fact that feeding with urea depressed appetite and increased the non-protein nitrogen of the blood. The latter effect probably causes a feeling of discomfort in animals. Depressed appetite did not appear to be due solely to the unpleasant flavour of urea in the food because the same effect was produced when animals were drenched with urea, and the larger the dose the greater the loss of appetite.

If the ill effects of urea could be overcome I believe its nutritive properties might be of value for ruminants and I hope that the discussion today may bring out methods of doing this. Meanwhile, urea is not being used in this country as a cattle food.

REFERENCE

Bartlett, S. and Cotton, A. G. (1938). *J. Dairy Res.* **9**, 263.

Sir Jack Drummond (Ministry of Food, Portman Court, Portman Square, London, W.1): Does urea pass into the milk to any appreciable

extent? Some of the discrepancies apparent from the discussion and also the finding that animals receiving urea do not thrive quite as well as those getting protein may well be related to the fact that protein contains other elements than nitrogen. Replacement of a large proportion of protein by urea might reduce appreciably the availability of sulphur.

Mr. W. S. Ferguson (Jealott's Hill Research Station, Bracknell, Berks.): American work suggests that the biological value of various proteins matters little in ruminant nutrition and thus offers some evidence that the biological value of the bacterial protein formed in the rumen may be high (Bratton, 1942; Miller and Morrison, 1942). On the other hand, work at the Hannah Dairy Institute suggests the existence of appreciable differences in the biological values of various vegetable proteins for dairy cows (Morris, Wright and Fowler, 1936). In these experiments the maintenance part of the ration consisted mainly of straw, which may not have supplied an entirely suitable medium for the growth of ruminal bacteria.

REFERENCES

Bratton, R. W. (1942). *Abstr. Thes. Univ. Cornell*, p. 455.
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Morris, S., Wright, N. S. and Fowler, A. B. (1936). *J. Dairy Res.* **7**, 97.

Dr. M. Pyke (Ministry of Food, Portman Court, Portman Square London, W.1): The possible importance of refection as a method of microbiological synthesis of protein in the rat has already been mentioned by Mr. Benesch. In this connexion I was interested in Mr. Baker's remark that bacteria found in the large intestine of rabbits which are known to eat their faeces are similar to the iodophile bacteria of ruminants. If it should be found that rabbits possess a microflora capable of synthesizing protein they would be more convenient for laboratory use than ruminants.

Mr. F. Baker (County Technical College, Guildford, Surrey): In the rabbit, the microbial population attains its highest density in the caecum. By the time the faeces have reached the lower regions of the colon and rectum a marked drop in density has occurred. This at least is the case with "day" faeces. I have not yet had occasion to examine the soft night faeces carefully, and cannot, therefore, make any statement about the density of their microbial population or the influence of coprophagy. I might, however, mention that, in addition to the supposed digestion in the stomach of the bacterial products of the faeces eaten, there is another route by which bacteria are normally eliminated, that is through the lymphoid tissues of the appendix (Baker and Enticknap, 1943). In this organ there is a hypertrophy of the macrophage components of the reticulo-endothelial system. By Romanowsky Wolbach staining I have demonstrated that large numbers of bacteria are ingested by the macrophages. That these bacteria have found their way in from the gut by penetrating the mucosa is clear from the fact that with iodine they give a strong blue reaction; they are thus iodophile species. I am engaged on work which will enable me to compute approximately the total bacterial content of the appendix. In the absence of fuller data, however, it would be altogether premature to assert that this route of assimilation

possesses biological significance for the animal as a means of endocellular digestion.

REFERENCE

Baker, F. and Enticknap, J. (1943). *Nature, Lond.*, **151**, 532.

Professor J. R. Marrack (London Hospital, Whitechapel, London, E.1): Has urea been estimated in the blood of cows whose diet contains this substance?

Dr. G. Fraenkel (Biological Field Station, London Road, Slough, Bucks.): Ruminants cannot increase their protein supplies by digesting bacteria because bacterial protein must be largely derived from the plant protein ingested and some loss in this conversion is inevitable. Is it economical, even under war conditions, to produce and give to cattle large quantities of ammonia?

Dr. J. A. B. Smith replied:

To Mr. Benesch: 1. The form in which non-protein nitrogen is given is undoubtedly very important. As Mr. Benesch has said it should become available in the rumen as gradually as possible, and some readily available carbohydrate should be ingested with it to supply the needs of the bacteria which are responsible for its conversion into protein. This may be one of the great advantages of the urea potato flakes or urea sugar beet slices used in Germany and of the ammoniated beet pulp which has been tested successfully in America (Millar, 1944).

2. If ammonium alginate is readily available it should certainly be tested as soon as possible.

3. The observation that, in the growth experiments which have been cited, urea was slightly inferior to its nitrogen equivalent of protein, is probably due in part to the fact that no matter how the N.P.N. is given a proportion of it is almost bound to pass from the rumen and be absorbed into the blood before it has been used by the bacteria. Some of this urea will pass from the blood stream back into the rumen through the saliva, but the remainder will be excreted in the urine. On the average, 25 per cent. of the dietary urea appeared to be excreted unused in the experiments at the Hannah Institute (Owen, Smith and Wright, 1943).

4. There are 3 possible reasons why urea has not been used in feeding stuffs in this country during the war. (*a*) The shortage of feeding stuffs in this country has not been confined to protein but has involved carbohydrate also. As already indicated the addition of N.P.N. to feeding stuffs deficient in carbohydrate would be valueless. (*b*) Due to the greatly increased use of urea for plastics, it is probable that there has been almost a world shortage of urea during the past few years. (*c*) The negative results which Dr. Bartlett has just quoted were available to the authorities though they were not published, and they would obviously discourage the use of urea in feeding stuffs.

5. Experiments with sulphonamides may very well help to elucidate some of the outstanding problems.

To Dr. Bartlett: The work referred to by Dr. Bartlett was not cited since reference was confined to published work. As already stated, if urea is to be utilized effectively it must become available in the rumen

as slowly as possible; adequate supplies of readily available carbohydrate must be given simultaneously and the urea should not supply more than about 30 per cent. of the total nitrogen of the diet. If these conditions were fulfilled in the work cited by Dr. Bartlett, the negative results obtained must at present remain unexplained.

There is, of course, no value in adding N.P.N. to a diet in which the amount of protein is already normal.

In no other experiments of which I am aware, and many have been described in recent years, could any harmful effect be traced to urea, provided it did not supply more than 40 per cent. of the nitrogen of the diet. At the Hannah Institute and in the American experiments, those of Hastings (1944) for example, urea had no harmful effect and the diets containing urea appeared to be eaten with relish.

Urea will naturally be toxic if given as a drench because it will be converted very rapidly indeed to ammonia in the rumen (Pearson and Smith, 1943) and so may well be harmful, but it is not suggested that urea should ever be given in this way. When the urea is given with the usual dietary constituents, the ammonia is formed more slowly and is neutralized by the lower fatty acids which are formed at the same time from the carbohydrate. The ill effects which Dr. Bartlett has mentioned can readily be overcome and, judged by the Hannah Institute experiments and by the literature of the last few years, they have rarely, if ever, been observed by the majority of workers.

To Sir Jack Drummond: In the experiments at the Hannah Institute the urea content of the milk secreted during urea administration never exceeded 0·028 per cent. or 0·16 g. per pint (Owen, Smith and Wright, 1943).

Provided N.P.N. supplies only 25 or 30 per cent. of the total nitrogen the risk of other elements being deficient will probably be small since bacteria can probably utilize inorganic forms of sulphur and other elements. This point is, however, very important and requires further investigation.

To Mr. Ferguson: The whole question of the biological value of proteins for ruminants is undoubtedly greatly complicated by the changes which all the dietary constituents may undergo in the rumen and may also depend on the nature of the dietary constituents other than the protein which is being tested. There are so many factors involved that much more detailed work is required on the whole subject before many of the points will be elucidated.

To Professor Marrack: Blood urea was estimated in the work at the Hannah Institute (Owen, Smith and Wright, 1943). The values for 5 cows averaged about 12 mg. urea per 100 ml. of blood for all the blood meal periods as compared with 18 mg. urea per 100 ml. for all the urea periods. The highest value obtained during a urea period was 26·9 mg. per 100 ml. of blood, and this was an exceptionally high figure.

References

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Millar, H. C. (1944). *J. Dairy Sci.* **27**, 225.
Owen, E. C., Smith, J. A. B. and Wright, N. C. (1943). *Biochem. J.* **37**, 44.
Pearson, R. M. and Smith, J. A. B. (1943). *Biochem. J.* **37**, 148.