DDP-substituted N-terminal amino acid splits off with such ease that mild hydrolytic conditions may be used under which the hydantoins are perfectly stable. Furthermore, as under these conditions the remaining peptide bonds are split only to a very small extent, a possible catalytic effect of the degradation products of certain amino acids on the stability of the marked N-terminal amino acids is eliminated.

Mixtures of the deeply coloured DDP-hydantoins can easily be resolved by adsorption chromatography; the isolated DDP-hydantoins can be identified not only by their chromatographic behaviour and their melting point but also by alkaline hydrolysis to regenerate the free amino acid. Paper chromatography can then provide an additional check of identity.

DDPI is thus an excellent reagent for the determination of the N-terminal amino acid residue in a peptide.

**SUMMARY**

1. A red-coloured isocyanate, the 4-dimethylamino-3:5-dinitrophenyl isocyanate (briefly called DDPDI) was synthesized.

2. It was found that this isocyanate reacts quantitatively with the free amino groups of amino acids or peptides dissolved in water-dioxan at the apparent pH 9-6 at 0°C, giving deep-orange coloured carbamyl derivatives (briefly called DDP-ureido amino acids or DDP-ureido peptides, for example, DDP-ureido glycine).

3. DDP-ureido-amino acids when refluxed for 2 hr. in 2N-hydrochloric acid, or when treated for several days at 40°C with nitromethane saturated with hydrochloric acid, give by ring closure and elimination of water the corresponding hydantoins (briefly called amino acid-DDP-hydantoin) in quantitative yields.

4. When DDP-ureido peptides are treated in the same way, the N-terminal amino acid carrying the DDP radical splits up, forming by ring closure the corresponding hydantoin which can be recovered in quantitative yields.

5. The amino acid-DDP-hydantoins are deep-orange coloured substances which crystallize well and have sharp melting points. They can be adsorbed on alumina columns, and their mixtures can be conveniently resolved by adsorption chromatography.

6. The scope for the use of these derivatives for the determination of N-terminal amino acids in peptides is discussed.

The authors wish to thank the Royal Society for a grant towards this investigation. One of us (G.G.E.) is indebted to the Monsanto Chemical Co. Ltd. for a personal grant.

**REFERENCES**


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**Studies on the Determination of the Sequence of Amino Acids in Peptides and Proteins**

4. **THE SYNTHESIS OF 3-(4'-DIMETHYLAMINO-3':5'-DINITROPHENYL)-2-THYOHYDANTOIN DERIVATIVES OF VARIOUS AMINO ACIDS, AND THEIR USE FOR AMINO ACID-SEQUENCE DETERMINATIONS**

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(Received 5 May 1953)

The use of isocyanates and isothiocyanates for the determination of the sequence of amino acids in peptides was discussed in a previous paper (Evans & Reith, 1954). Aschan (1883) synthesized thiohydantoins by heating phenyl isothiocyanate with glycine, alanine and leucine at 140°C. The method of forming the thiohydantoins acids in alkaline solution and subsequently obtaining the thiohydantoins by acid catalysis was first used by Marckwald, Neu- mark & Stelzner (1891). The difficulty experienced in quantitative separation and estimation of mixtures of thiohydantoins, when only small quantities of substance are available, has led us to the synthesis of coloured thiohydantoins. Their preparation and use for the determination of the sequence of amino acids in peptides is described in

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this paper. The abbreviated nomenclature used is similar to that proposed for the isocyanate derivatives (Evans & Reith, 1954). A short account has already been communicated to the Biochemical Society (Reith & Waldron, 1953).

EXPERIMENTAL AND RESULTS

Preparation of 4-dimethylamino-3:5-dinitrophenyl isothiocyanate (DDPT)

N-Acetyl-4-dimethylamino-3:5-dinitroaniline. 4-Dimethylamino-3:5-dinitrobenzoyl azide was prepared by the method already described (Evans & Reith, 1954). The azide (9-85 g.) was added to a mixture of acetic anhydride (30 ml.) and conc. H₂SO₄ (3 ml.) contained in an apparatus as described for the preparation of DDPI (Evans & Reith, 1954), and the air was displaced by CO₂. The temperature of the bath was slowly increased to 85° during 25 min., and after a further 40 min. the temperature had reached 90° when the reaction was complete. The amount of nitrogen evolved was 775-0 ml. (0°/760 mm.), theory requires 788 ml. After cooling to room temperature, acetic acid (30 ml.) was added, and the solution was then added slowly, dropwise to water (600 ml.) well stirred. The orange solid obtained was filtered off, washed with water and dried. Yield 8-72 g.; m.p. 180-184°.

The substance recrystallized from toluene (200 ml.), yielded N-acetyl-4-dimethylamino-3:5-dinitroaniline. 6-60 g. (70%), m.p. 200-201° (decomp.) (Found: C, 45-1; H, 4-5; N, 21-1. C₁₀H₁₃N₃O₅ requires C, 44-8; H, 4-5; N, 20-9%.) Another 1-01 g., m.p. 170-185°, was obtained from the mother liquors.

4-Dimethylamino-3:5-dinitroaniline. The N-acetyl derivative (7-17 g.) was added to a solution of water (20 ml.), conc. H₂SO₄ (10 ml) and acetic acid (20 ml.) and was heated in a boiling-water bath for 1 hr. The cooled solution was poured into water (300 ml.) when a thick orange precipitate of the amine sulphate appeared, but on addition of excess of conc. NH₄OH this was converted into a deep-red precipitate of the amine. The crude amine was filtered off, washed with water, immediately dissolved in 6x-HCl (1200 ml.) and filtered from a small amount of impurity. The acid solution was neutralized with conc. ammonia when the amine appeared as a deep-maroon precipitate (5-6 g., 93-5% yield). This was dissolved in boiling CCl₄ (300 ml.), treated with charcoal and filtered while hot. The filtrate was concentrated (130 ml.) and on cooling red crystals of 4-dimethylamino-3:5-dinitroaniline were obtained, yield 5-04 g. (83%), m.p. 155-156°. Another 0-34 g was obtained from the mother liquor. (Found: C, 42-5; H, 4-7; N, 24-7. C₁₀H₁₃N₃O₅ requires C, 42-5; H, 4-5; N, 24-8%.) The step in the purification when the amine is dissolved in 6x-HCl is particularly important in obtaining a good product though the amount of impurity removed is very small. The amine crystallized in needles having two distinct colours, deep maroon and orange-red. The former are the more stable at higher temperatures and consequently precipitated first from the CCl₄.

4-Dimethylamino-3:5-dinitrophenyl isothiocyanate (DDPT). Thiophosgene (2-64 g.; b.p. 72-5-73°) was weighed into a stoppered flask containing water (10 ml.) More water (25 ml.) was added together with CHCl₃ (25 ml.), and the mixture was stirred at 0° whilst 4-dimethylamino-3:5-dinitroaniline (4-18 g.) was added in CHCl₃ (80 ml.) during 30 min.; the mixture was stirred for a further hour at 0°, and then 1 hr. at 20°. The CHCl₃ layer was separated off, and the water solution extracted with two small amounts (10 ml.) of CHCl₃. The combined CHCl₃ solutions were dried overnight with CaCl₂ when a few yellow needles of the amine hydrochloride separated. After filtering, the CHCl₃ solution was evaporated in vacuo and the residue dried, yield 4-94 g. (99-5%), m.p. 100-103°. The 4-dimethylamino-3:5-dinitrophenyl isothiocyanate (DDPT) was recrystallized from light petroleum, b.p. 60-80°, yield 4-58 g. (92-5%), m.p. 103-104°. (Found: C, 40-7; H, 2-9; N, 20-8; S, 11-8. C₁₅H₁₁N₅O₅S requires C, 40-3; H, 3-0; N, 20-9; S, 11-9%.) Additional material was obtained from the mother liquors, yielding the total yield to 97-5%. The orange-red flat needles were soluble in ether, acetone, benzene, CHCl₃, CCl₄, ethyl acetate, dioxan and hot light petroleum.

Reaction of DDPT with aniline. To characterize the DDPT, redistilled aniline (31 mg.) in ether (3 ml.) was treated with DDPT (89 mg.) when a red solid precipitated. The mixture was evaporated in vacuo, and the residue (117 mg.; m.p. 176-178°) was recrystallized from ethanol when red crystals of N-(4-dimethylamino-3:5-dinitrophenyl)-N`-phenylthiourea m.p. 180-181° were obtained. (Found: C, 50-1; H, 4-0; N, 19-6; S, 9-0. C₁₅H₁₃N₅O₅S requires C, 49-9; H, 4-2; N, 19-4; S, 8-9%)

Reaction of DDPT with amino acids and peptides

The reaction was carried out either in a NaHCO₃:water:acetic solution at a pH reading of 8-5 (glass electrode), or better in a sodium acetate:water:acetone solution at a pH reading of 8-0, which was increased at the end of the reaction to pH 9-0 by addition of alkali; in these solutions containing a large amount of acetone the recorded pH is only nominal. Sodium acetate (136 mg.) in water (6 ml.) gave a pH value of 7-3 (probably due to CO₂ in the water used); on adding acetone (12 ml.) the pH reading became 8-9 but the solution is actually more or less neutral.

Glycine (37-5 g.; 0-5 mmole) in water (6 ml.) containing sodium acetate (136 mg.) was stirred with acetone (12 ml.), and DDPT (147 mg.; 0-55 mmole) in acetone (6 ml.) was added all at once. The pH was constantly controlled and 0-5x-NaOH was added to keep the value at 8-0 for 40 min. when the pH was raised to 9-0 for 30 min. The theoretically required amount of 1-00 ml. of alkali was needed. Evidently, when in the secondary reaction the DDPT reacts with water forming the symmetrical thiourea, the carbon oxysulphide produced is not acidic enough to titrate with alkali at the pH used. After a total period of 70 min. reaction time the solution was concentrated in vacuo to remove acetone, diluted with water (15 ml.) and the precipitated excess of DDPT and the symmetrical thiourea (m.p. 195-196°) were three times extracted with ether (10 ml.). The ether dissolved in the water was removed in vacuo, and the DDPT-glycine precipitated by a very slow addition of 0-5x-NaOH (3 ml.); yield 158 mg. (92%). A further amount of substance was obtained from the mother liquor, increasing the total yield to 99%. Recrystallization from acetone by slow addition of light petroleum, b.p. 40-60° gave orange crystals of 4-dimethylamino-3:5-dinitrophenylthiocarbamyl-glycine (DDP-thioureidoglycine), m.p. 158-159° (decomp.).

The NN`-bis-(4-dimethylamino-3:5-dinitrophenyl)thiourea formed as by-product by the action of water on DDPT was isolated and identified; m.p. 194-195°. (Found: C, 41-2; H, 3-7; N, 21-8; S, 6-2. C₁₅H₁₁N₅O₅S requires C, 41-4; H, 3-7; N, 21-6; S, 6-5%.)
Table 1. *DDP-thioureidoamino acids and DDP-thioureidopeptides prepared by the method given in the text* 

<table>
<thead>
<tr>
<th>Derived from</th>
<th>Yield (%)</th>
<th>M.p. (°C)</th>
<th>Found (%)</th>
<th>Requires (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>99</td>
<td>158-159</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>DL-Alanine</td>
<td>100</td>
<td>145-149</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>94</td>
<td>90-110</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>L-Lysine (di-DDP)</td>
<td>99</td>
<td>95-115</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Glycylglycine</td>
<td>99</td>
<td>185-186</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DL-Alanylglucylglycine</td>
<td>96</td>
<td>132-135</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

|                        |          |           |           |           |
|                        |          |           | C         | H         |
|                        |          |           | H         | N         |
|                        |          |           | S         | S         |

* All melting points are corrected.

Table 2. *Amino acid-DDP-thiohydantoins prepared by the method given in the text*

<table>
<thead>
<tr>
<th>Derived from</th>
<th>Yield (%)</th>
<th>M.p. (°C)</th>
<th>Found (%)</th>
<th>Requires (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>91</td>
<td>253-254</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>DL-Alanine</td>
<td>87</td>
<td>222-223</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>88</td>
<td>219-220</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>77</td>
<td>219-220</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

|                        |          |           |           |           |
|                        |          |           | C         | H         |
|                        |          |           | H         | N         |
|                        |          |           | S         | S         |

* All melting points are corrected.

The data of the derivatives which we have prepared are given in Table 1.

In the preparation of di-DDP-thioureidolysine, the precipitation with 0.5 N HCl gave a deep-red precipitate which was almost colloidal. This was three times extracted with ethyl acetate, the ethyl acetate solution was washed with water and evaporated in vacuo (<20°C). The solid was dried in vacuo over H₂SO₄.

**Preparation of DDP-thiohydantoins**

The thiohydantoins were prepared from the DDP-thioureido-amino acids by treatment with either glacial acetic acid or with acetic acid: water (20:80, v/v) at about 40° for 5 days. As an example the preparation of 3-(4'-dimethylamino-3'-5'-dinitrophenyl)-2-thiohydantoin (glycine-DDP-thiohydantoin) is described. DDP-thiohydantoin glycine (50 mg) was heated for 5 days in glacial acetic acid (3 ml) at 40° and evaporated in vacuo. The residue was dissolved in acetone (1 ml) and benzene (7 ml) was added. This solution was placed on a 20 g. alumina column (10 x 220 mm.), and the chromatogram was developed with benzene: methanol (99:76:0.26, v/v; 76 ml). The main zone was eluted with acetone and the eluent evaporated in vacuo; yield, 43.3 mg. (91%) of an orange crystalline substance, m.p. 253-254° (decomp.). The DDP-thiohydantoins prepared are listed in Table 2.

In the case of the phenylalanine-DDP-thiohydantoin the substance eluted from the column and crystallized contained benzene (probably 0.5 mole solvent of crystallization). The benzene was removed by dissolving the solid in acetone and evaporating the solution to dryness.

The hydantoins are deep-orange crystalline substances; the optically active amino acids were extensively racemized.

**Regeneration of amino acids from their DDP-thiohydantoins**

Wheeler & Hoffmann (1911) found that when hydantoins were refluxed for 5 hr. in barium hydroxide solution some 75% of the amino acids were obtained. Edman (1850) used this method in the case of the phenylthiohydantoin derivatives, and identified the amino acids by paper chromatography. We found that in the case of the DDP-thiohydantoins acid hydrolysis was preferable to the alkaline treatment. It seems that in alkaline solution the sulphur is quickly removed and the barium sulphide thus produced reacts with the nitro groups giving amino compounds. From a number of acids investigated a mixture of formic acid, acetic anhydride and perchloric acid which was used by Hanes, Hird & Isherwood (1952) for the hydrolysis of peptide bonds has proved to be very convenient. The reaction was carried out in the following way: alanine-DDP-thiohydantoin (5 mg) was heated with 1 ml. of a mixture of formic acid (90%, w/v; 10 vol.), acetic anhydride (5:5 vol.) and perchloric acid (60%, w/v; 1:5 vol.) for 1 day at 120°. The solution was brought to pH 3 (indicator paper) by the addition of N-KOH, and was then evaporated by standing in a desiccator over NaOH. The alanine was extracted with ethanol: water (50:50, v/v), the potassium perchlorate being filtered off. Some of this solution was placed on a strip of filter paper and the chromatogram was developed in the usual way with a n-butanol: acetic acid: water mixture.

**Spectrophotometric estimations**

The absorption measurements were carried out in ethanol at concentrations between 0.1 and 1.6 x 10⁻⁴ M for measurements at the 410 m. maximum, and between 0.1 and 1.6 x 10⁻⁴ M for measurements at the other maxima (1 cm.
### Chromatographic separation of the DDP-thiohydantoins

The alumina was prepared by the method already described (Flowers & Reith, 1953). As an example, the separation of a mixture of glycine-DDP-thiohydantoin with alanine-DDP-thiohydantoin proceeds as follows. Glycine-DDP-thiohydantoin (2.9 mg.) and alanine-DDP-thiohydantoin (2.2 mg.) were dissolved in acetone (0.2 ml.) and benzene (1.0 ml.) was added. This solution was adsorbed on a 20 g. alumina column (10 × 350 mm.) made with a slurry with benzene. The column was developed with benzene: methanol (99:9:0-1, v/v, 45 ml.), until the appearance of the chromatogram was 0-39 mm. white, 39-84 mm. orange, 64-78 mm. white, 78-95 mm. orange. The coloured zones were scraped out and eluted with acetone. The acetone solutions were evaporated in vacuo, the residues dissolved in ethanol and estimated spectrophotometrically at 410 m\u21a6 maximum; yield of the upper zone (glycine-DDP-thiohydantoin) 2-28 mg. (99-6\% recovery); yield of the lower zone (alanine-DDP-thiohydantoin) 2-19 mg. (99-5\% recovery).

The removal of N-terminal amino acids as DDP-ureidopeptides. DDP-thioureaalanine-DAPA (50 mg.) was shaken at 42-5\°C for varying periods of time with acetic acid: water (20:80, v/v; 3 ml.). The mixture was then evaporated in vacuo, the residue was shaken with acetone (2 ml.) for 1 hr. after which benzene (10 ml.) and water (10 ml.) were added and the solute partitioned between these solvents. The water layer was kept for investigation. The combined benzene extracts were evaporated in vacuo, the residue dissolved in acetone (1 ml.), diluted with benzene (7 ml.) and adsorbed on a 20 g. alumina column. The column was developed with benzene:methanol (99:75:0-25, v/v; 100 ml.). The appearance of the column in the ease of the 5-day experiment was 0-1 mm. deep red, 1-4 pale brown, 4-17 very pale yellow, 17-21 yellow, 21-35 pale yellow, 35-39 pale pink, 39-110 white, 110-240 yellow increasing to orange. The top zones were scraped out. The main zone (110-240 mm.) was eluted with acetone, the acetone evaporated in vacuo and the residue consisting of glycine-DDP-thiohydantoin was estimated gravimetrically; the yields were as follows. 2 days 19-9 mg. (49\%), 2-75 days 26-2 mg. (64-5\%), 3 days 29-8 mg. (73\%), 4 days 35-3 mg. (87\%), 5 days 37-6 mg. (92-5\%). The water layer (see above) which contained glycine was titrated with alkali by the formol titration method (glass electrode). For the 2-75-day reaction the yield of glycine was 6-3 mg. (67\%), for the 5-day reaction 9-1 mg. (97\%).

An experiment with DDP-thioureaalanine-DAPA (50 mg.) carried out in a similar way gave the following yields of alanine-DDP-thiohydantoin: in the experiment lasting 1 day 30-6 mg. (85\%), 2 days 30-6 mg. (85\%), 4 days 31-0 mg. (86\%). The water layer containing the glycine-DDP-thiohydantoin was in each case titrated with alkali by the formol method. For the 1-day reaction the yield of glycine was 11-7 mg., 2 days 11-5 mg., 4 days 12-4 mg. (the mean is 85\%), which corresponds quite well with the quantity expected from the 85\% yield of alanine-DDP-thiohydantoin.

### Sequence determination in alanlyglycylglycine

Alanylglucine (20-3 mg.) was treated with DDPT (40 mg.) as described above. After acidifying the water solution with 0-5N-HCl, the DDP-thioureaalanine-DAPA (50 mg.) was extracted with ethyl acetate (5 ml.) five times. The combined ethyl acetate extracts were evaporated in vacuo, and the residue was shaken with water:acetic acid (80:20, v/v; 3 ml.) for 1 day at 42-5\°C in a ‘Quickfit’ flask (50 ml.) closed with a cone carrying a tap. The cone was strongly held in position by steel springs, and before shaking, the flask was evacuated to 100 mm. Hg. The solution was evaporated in vacuo, acetone (2 ml.) was added and the mixture was shaken for 1 hr.; benzene (10 ml.) and water (10 ml.) were then added and the solute partitioned between these two solvents. The benzene solution was evaporated in vacuo, the residue dissolved in acetone (1 ml.), diluted with benzene (7 ml.) and adsorbed on a 20 g. alumina column. The column was developed with benzene:methanol (99:9:0-1, v/v); after separation the upper bands were scraped out, and the main zone eluted with acetone. The acetone solution yielded 28-0 mg. (82-5\%) of alanine-DDP-thiohydantoin which was identified by its melting point and by paper chromatography.

The water solution, which contained glycine-DAPA, was then treated in an analogous manner. The yield of the first glycine-DDP-thiohydantoin was 23-3 mg. (71-8\% of yield calc. for alanlyglycylglycine), and that of the second was 19-7 mg. (60-5\%).

### DISCUSSION

Although 4-dimethylamino-3:5-dinitrophenyl isocyanate is an excellent reagent for the determination of N-terminal amino acids (see preceding paper), it was not entirely satisfactory for the determination of the sequence of amino acids by successive applications to a peptide. The use of DDPT described in this paper overcomes this difficulty. It gives excellent yields of DDP-thioureaaminoacids and peptides under mild conditions, and these are converted into DDP-thiohydantoins under much milder conditions than were necessary for the DDP-ureidopropionic acids and DDP-ureidopeptides.

The DDP-thiohydantoins are deeply coloured compounds which can be separated from one another by chromatography and can easily be estimated by colorimetric techniques. By subsequent acid hydrolysis of these thiohydantoins the parent free amino acid can be regenerated and its identity can be checked by paper chromatography.
DDPT is thus a suitable reagent for the determination of the amino-acid sequence in peptides by a process of step-wise degradation from the N-terminal end of the chain, and has in fact been successfully applied for this purpose. The conditions under which the N-terminal amino acid splits off as a substituted DDP-thiohydantoin are so mild that other peptide bonds are not split. The yields of the thiohydantoins vary between 80 and 90% according to the experimental conditions and the type of the thiohydantoin. We found that the yield of the residual peptide (subject to the next step in the sequence determination) is practically equivalent to the yield of the thiohydantoin. Consequently a small but calculable fraction of the DDP-thioureidopeptide is lost during the process of the elimination of the substituted N-terminal group.

SUMMARY

1. A deep-orange isothiocyanate, 4-dimethylamino-3:5-dinitrophenylisothiocyanate (briefly called DDPT) was synthesized.

2. It was found that this isothiocyanate reacts with amino acids or peptides dissolved in water: acetone (1:3, v/v) at the apparent pH 8-9, giving orange thio carbamyl derivatives (briefly called DDP-thioureidoamino acids or DDP-thioureidopeptides, e.g. DDP-thioureidoglycine) in practically quantitative yields.

3. By the treatment of the DDP-thioureidoamino acids with glacial acetic acid (in the case of DDP-thioureidoglycine) or with water: acetic acid (in the case of other DDP-thioureidoamino acids) the corresponding substituted 2-thiohydantoins (briefly called amino acid-DDP-thiohydantoins, e.g. glycine-DDP-thiohydantoin) are formed in good yields (80-90%).

4. By treatment of DDP-thioureidopeptides with water: acetic acid, the N-terminal amino acid residue splits off as an amino acid-DDP-thiohydantoin which can be isolated in a pure state by adsorption chromatography; the residual peptide can be treated with DDPT and the process repeated to determine the sequence of amino acids in the peptide.

5. As an example the sequence determination on alanylglycylglycine is described.

6. The amino acid-DDP-thiohydantoins can be hydrolysed to the parent amino acids and these can be identified by the use of paper chromatography.

The authors wish to thank the Royal Society for a grant towards this investigation. One of us (N.M.W.) is indebted to the Department of Scientific and Industrial Research for a personal grant.

REFERENCES


The Extent of Conversion of Food Protein to Microbial Protein in the Rumen of the Sheep

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Interest in the role of ruminal micro-organisms in the protein metabolism of ruminants stems from the hypothesis of Zuntz (1891) that these organisms might utilize dietary non-protein nitrogen (N.P.N.) for their growth, with resultant synthesis of protein which would become available to the host. Numerous experiments have established the validity of this view (for a review of the extensive literature in this field see McNaught & Smith, 1947); indeed, Loosli, Williams, Thomas, Ferris & Maynard (1949) were able to maintain growth in sheep on a diet in which virtually all the nitrogen was supplied by urea.

In spite of the activity in this field, comparatively little attention has been given to the possibility of microbial digestion of food protein in the rumen. In normal ruminant diets most of the nitrogen occurs in the form of protein. The facts that secretory glands do not occur in the rumen, that the saliva contains no proteolytic enzyme (Wegner, Booth, Bohstedt & Hart, 1940) and that the rumen contents are strongly proteolytic (Sym, 1938) suggest that ruminal organisms play an active part in the digestion of protein. Pearson & Smith (1943) observed breakdown of protein by rumen liquor in