Degradation of nucleic acids in the rumen

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1. Nucleic acids introduced into the rumens of calves, or incubated with calf, sheep or cow rumen contents in vitro, were rapidly destroyed.

2. The degradation products formed were separated and identified by means of column chromatography on Sephadex G-10 Dextran gel and thin-layer chromatography on cellulose.

3. In vitro, RNA was rapidly (within 1 h) converted into ultrafilterable oligo-and mono-nucleotides, nucleosides, purine and pyrimidine bases. After 4 h, only the bases xanthine, hypoxanthine and uracil remained, having increased at the expense of the other constituents.

4. DNA gave similar products but with a much greater proportion of ultrafilterable oligo- and mono-nucleotide material which remained as a major component even after 4 h. The only bases present in appreciable amounts were thymine, hypoxanthine, uracil and xanthine.

5. The same products accumulated temporarily in vivo, after addition of RNA or DNA to the rumens of calves, and were found also, in small amounts, in corresponding samples of duodenal digesta. The products disappeared from the rumen at a greater rate than could be accounted for by transfer to the duodenum.

6. Cell-free preparations from calf rumen fluid contained enzymes which converted RNA and DNA into products which appeared to be ultrafilterable oligonucleotides.

7. When ground hay was incubated with whole rumen contents the nucleic acids in the hay were degraded to a mixture of nucleotides, nucleosides and bases, almost as readily as were pure nucleic acids.

Enzymes capable of degrading both RNA (Privat de Garilhe, 1967; Laskowski, 1968; Barnard, 1969) and DNA (Kurnick, 1962) have been isolated from many species of bacteria. Details of modes of action and specificities of some have been published (see Cantoni & Davies, 1966). There is very little information available on rumen nuclease activities but, with the large mixed microbial populations, various organisms capable of acting upon both RNA and DNA might be expected to be present. Germanyuk (1964) reported ribonuclease activity in the rumen contents of cows, and Smith & McAllan (1970*a*) found that when RNA or DNA were added to the rumen of calves, or were incubated in vitro with calf rumen contents, they were rapidly destroyed.

To study the breakdown of RNA and DNA by mixed rumen bacteria, a method of separating and identifying the numerous possible end-products was required. Gelotte (1960) first reported the reversible binding of several purines and pyrimidines on Sephadex G-25 Dextran gel, and De Bersaques (1967) showed that the effect was enhanced with the more tightly cross-linked Sephadex G-10. The exact mechanism of this binding is not known, but Sweetman & Nyhan (1971) reported that purines interact primarily with the Dextran moiety of the gel. The combined binding and molecular-sieving properties of Sephadex have been used in the present study. A preliminary report on part of this work has been published previously (Smith & McAllan, 1970*b*).

MATERIALS AND METHODS

Animals and feeding. Most of the work was carried out with castrated male Friesian calves, aged 15–39 weeks, but a Friesian cow, 3·5 years old and a crossbred wether sheep, 1·5 years old, were also used. All animals were equipped with rumen cannulas and some of the calves also had a simple duodenal cannula. These preparations have been described previously (Smith & McAllan, 1970*a*).

Some experiments were carried out with calves which were allowed to graze pasture, but most experiments were with calves given either flaked maize and hay in equal proportions or a similar diet with added decorticated, extracted groundnut meal (diets A and B in Smith & McAllan, 1970*a*). The cow was given 5·0 kg hay and 5·0 kg dairy cubes (Smith & McAllan, 1970*a*)/d and the sheep 0·8 kg hay/d. Except for the calves at pasture, all animals were fed twice daily (06.30–09.00 hours and 16.30–17.00 hours).

Collection and treatment of digesta samples

In vivo experiments. Samples of rumen fluid were examined at different times after addition of nucleic acids to the rumen. These were added in solution and in some experiments the solution also contained polyethylene glycol (PEG) (mol. wt 4000) as a non-absorbable marker. Samples were sucked from the rumen through a tube of about 10 mm internal diameter. They were collected in a vessel surrounded by ice and were strained through surgical gauze. Within 15 min of collection a sample was subdivided; RNA and DNA were estimated in one part and the other was centrifuged for 5 min at 30000***g*** at 4°. The supernatant fraction was poured off and ultrafiltered through cellophane tubing of 6 mm diameter (Union Carbide International Co., New York) according to the procedure of Gregory (1954). Samples of abomasal effluent were obtained by allowing digesta to flow from a cannula in the proximal duodenum. Samples (about 100 ml) were generally obtained in 5–10 min.

In vitro experiments. Samples from the rumen were collected as described above. When experiments to study metabolic changes in the whole rumen fluid were to be carried out, about 300 ml were collected. A gas mixture, nitrogen and carbon dioxide (95:5, by volume), was immediately passed into the fluid for 2 min and the vessel was closed with a rubber bung. In most experiments 24 g samples of rumen fluid were transferred to 100 ml flat-bottomed, wide-necked flasks containing 1 ml of a solution of the substrate to be investigated. In some experiments 2 g ground hay were added to 25 ml rumen fluid in similar flasks. The flasks were closed with rubber bungs fitted with Bunsen valves and gas inlet tubes, placed immediately in a water-bath at 37° and the N_2–CO_2 gas mixture was passed into the fluid for 2 min. The flasks were sealed and shaken mechanically for varying periods of time up to 4 h, with further 2 min periods of passing the gas mixture at 30 min intervals. With this procedure the pH of the mixtures remained virtually the same as that of freshly collected rumen contents: within the range 6·8–7·1 throughout incubation.

When cell-free filtrate was to be examined, a cooled sample of rumen fluid was, as soon as possible (within 10 min of collection), transferred to centrifuge tubes and centrifuged at 30000***g*** for 15 min at 4°. The supernatant fraction was poured off and

filtered, under reduced pressure, through a membrane filter of 0·22 μm (Millipore (UK) Ltd, London). The resultant cell-free filtrate was stored at $-20°$ until required.

Chemicals

Nucleic acids prepared commercially with some degree of depolymerization (pure grade; Koch–Light Laboratories Ltd, Colnbrook, Bucks.) were used in many experiments. These will be referred to as yeast RNA, thymus DNA or herring-sperm DNA. Batches were examined before use and only materials with less than 10% ultrafilterable u.v.-absorbing material were used. For some experiments highly polymerized RNA (ex yeast) and highly polymerized DNA (ex thymus) (British Drug Houses Chemicals Ltd, Poole, Dorset) were used. Inosine, xanthine, hypoxanthine and uridine mononucleotide (2′,3′ mixed isomers) were also obtained from British Drug Houses. Xanthosine was obtained from Phasesep R. & D. Chemical Co., Queensferry, Flintshire and the adenylic acid oligonucleotide series (terminal 2′:3′ cyclic phosphate) from Miles Laboratories Inc., Indiana, USA. Other nucleic acids, bases, nucleosides and nucleotides were obtained from Koch–Light Laboratories Ltd.

All other chemicals used were analytical reagent grade.

Analytical methods

Nucleic acids. RNA and DNA were determined by the procedures of McAllan & Smith (1969).

Polyethylene glycol (PEG). This was estimated as described by Smith & McAllan (1970*a*) for rumen fluid and Smith & McAllan (1971) for duodenal digesta.

Substances produced by the degradation of RNA and DNA. These were separated and identified by chromatography on Sephadex Dextran gels (Pharmacia (GB) Ltd, London) with confirmatory identification of products by thin-layer chromatography. Columns (900 mm × 15 mm) of G-10 and G-25 Sephadex equilibrated with 0·01 M-ammonium acetate, pH 7·0, were used in preliminary experiments. Void volumes (V_0) were determined by the use of Blue Dextran 2000 (Pharmacia (GB) Ltd, London) and eluting with 0·01 M-ammonium acetate. To calibrate the columns, solutions of known nucleic acids, nucleotides, nucleosides and bases were prepared. Nucleic acids and bases were dissolved in a minimum volume of 0·01 M-sodium hydroxide and made up to the required volume with 0·01 M-ammonium acetate. Nucleosides and nucleotides were soluble in 0·01 M-ammonium acetate. Solutions were prepared containing 0·38–2·75 μmol substrate/ml. Samples of these solutions (2 ml) were added to the columns and the chromatograms were developed with 0·01 M-ammonium acetate, pH 7·0. Extinction at 254 nm was measured in the effluent with an ISCO 222 double beam u.v. analyser (Instrumentation Specialities Co. Inc., Nebraska, USA) equipped with 5 mm cells, and recorded continuously on a chart (speed 20 mm/h) on which an event marker also recorded the collection of 3 ml fractions. Results for the positions of the different substances are given in Table 1. These are expressed as partition coefficients (K_D) which are derived from the equation:

$$K_D = \frac{\text{elution volume} - \text{void volume}}{\text{total bed volume} - \text{void volume} - \text{gel matrix volume}} \qquad (1)$$

Table 1. *Comparison of partition coefficients* (K_D) *obtained for nucleic acid derivatives on columns of Sephadex G-25 and G-10 Dextran gels*

(0·75–5·5 μmol sample, in a volume of 2 ml, were applied to 900 mm × 15 mm columns of Sephadex G-25 and G-10 Dextran gels. Elutions were carried out under the conditions described on p. 333)

Substance	Sephadex G-25	Sephadex G-10
Adenine	2·20	6·11
Guanine	2·07	4·87
Xanthine	1·68	3·11
Hypoxanthine	nd	2·18
Cytosine	1·37	1·37
Uracil	1·27	1·67
Thymine	1·32	1·73
Adenosine	1·73	3·29
Guanosine	1·80	2·52
Xanthosine	nd	1·33
Inosine	nd	1·20
Cytidine	1·20	1·03
Uridine	1·11	1·07
Thymidine	1·10	1·07
Deoxyadenosine	1·56	3·28
Deoxyguanosine	1·45	2·25
Uric acid	1·95	3·00
DNA (thymus)	0·10	0·04
RNA (yeast)	0·10	0·00
Mononucleotides, 2′,3′ mixed isomers		
Adenosine monophosphate	1·32	0·79
Guanosine monophosphate	1·39	0·60
Cytidine monophosphate	0·93	0·60
Uridine monophosphate	0·93	0·31
2′:3′ cyclic adenosine phosphates, lithium salts		
A > p	1·55	0·77
ApA > p	1·55	0·77
$(Ap)_2A$ > p	1·28	0·75
$(Ap)_3A$ > p	1·11	0·69
$(Ap)_4A$ > p	0·89	0·71
$(Ap)_5A$ > p	0·70	0·71

nd, not determined; A, mononucleotide; ApA, dinucleotide; $(Ap)_2A$, trinucleotide; $(Ap)_3A$, tetranucleotide; $(Ap)_4A$, pentanucleotide; $(Ap)_5A$, hexanucleotide; > p indicates a terminal 2′:3′ cyclic phosphatyl group.

All, except the oligonucleotide series, were repeated at least six times in different mixtures, with little variation. Mean values are given.

For subsequent experiments the G-10 column was used because it gave better separation of derivatives. Many experiments were carried out in which the amounts of the substance added to the G-10 column were accurately measured and in different experiments different amounts were used (0·75–5·5 μmol). The peak areas were calculated (width (measured as a volume of eluate) × 0·5 height (extinction)) and related to the amounts applied to the column. There was a linear relationship between amounts applied and areas under the curves. As examples, results for adenosine and guanine are shown in Fig. 1. Relative areas for different materials are shown in Table 2.

Two-dimensional chromatography was performed on 0·5 mm layers of MN 300 G

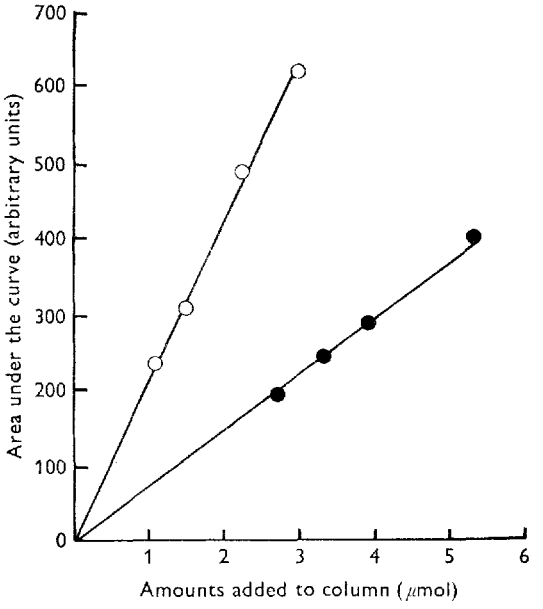

Fig. 1. Relationships between eluted peak areas and concentrations of materials chromatographed on Sephadex G-10. Samples (2 ml) of solutions containing adenosine (○) or guanine (●) were applied to a 900 mm × 15 mm Sephadex G-10 column and eluted under conditions described on p. 333.

Table 2. *Relative areas of derivative peaks eluted from G-10 column*

(Areas (derived as described on p. 334) obtained per μmol of derivative applied to a Sephadex G-10 column, are expressed relative to adenosine which has been given an arbitrary value of 1)

Derivative	Relative area/μmol
Adenine	0·81
Guanine	0·35
Xanthine	0·44
Hypoxanthine	0·73
Uracil	0·57
Thymine	0·44
Cytosine	0·37
Adenosine	1·00
Guanosine	0·84
Xanthosine	0·65
Inosine	0·70
Uridine	0·61
Thymidine	0·54
Cytidine	0·51
Adenylic acid (2′,3′) (mixed)	0·65
Cytidylic acid (2′,3′) (mixed)	0·43
Uridylic acid (2′,3′) (mixed)	0·39
Guanylic acid (2′,3′) (mixed)	0·57

Table 3. R_F *values obtained for the derivatives shown during two-dimensional thin-layer chromatography with water or* n-*butanol–water as solvent*

(Preparation of plates and conditions of development were as described below. 100 μl solution containing 0·25–0·50 μmol substances were applied to the plates)

Substance	Water	*n*-Butanol–water
Guanosine	0·66	0·22
Hypoxanthine	0·65	0·44
Adenosine	0·60	0·33
Xanthine	0·48	0·33
Uric acid	0·47	0·14
Xanthosine	0·58	0·24
Inosine	0·85	0·22
Cytosine	0·66	0·22
Uracil	0·79	0·47
Thymine	0·73	0·58

cellulose (Macherey, Nagel & Co., Düren, Germany) spread on 200 mm × 200 mm glass plates. The samples examined were column fractions, combined for individual peaks, and concentrated under reduced pressure. Volumes of approximately 200 μl, containing approximately 75 μg of a derivative, were applied to the plates. The plates were developed in the first direction, with distilled water, for 1 h, and in the second direction, with *n*-butanol–water (86:14, by volume) for 4 h. The spots were detected under u.v. light at 260 nm, and identification was by comparison of R_F values with those of known derivatives (Table 3) and by chromatographing samples with known derivatives.

RESULTS

Degradation of nucleic acids in vitro. Rates of disappearance of samples of yeast RNA and thymus DNA, incubated with whole rumen contents from calves, were reported by Smith & McAllan (1970*a*). In the present investigation similar results were obtained with rumen contents from the cow and sheep (Fig. 2) as well as in experiments with rumen contents of calves receiving pasture or flaked maize and hay (diet A) sometimes with the addition of decorticated, extracted groundnut meal (diet B). In all experiments RNA was virtually completely degraded after only 1 h. On the other hand, DNA was degraded more slowly so that even after 4 h appreciable amounts ($36 \pm 3\%$ in eight experiments with calves, sheep and cow) of the original material remained. Disappearance of nucleic acids in experiments with calf rumen contents was accompanied by the appearance of materials which were ultrafilterable (permeable through 6 mm Visking cellophane tubing, mol. exclusion limit approximately 6000), and showed u.v. absorption spectra characteristic of nucleic acids and their derivatives (cf. McAllan & Smith, 1969). The results given in Fig. 3 are typical examples of results obtained in experiments with calves either grazing pasture (two experiments) or receiving flaked maize and hay (two experiments). They show the amounts of ultrafilterable substances expressed in terms of concentrations of nucleic acids (mg/ml) which would show equivalent u.v. absorption at 260 nm, and these appear to be approximately the same as the amounts of nucleic acids disappearing.

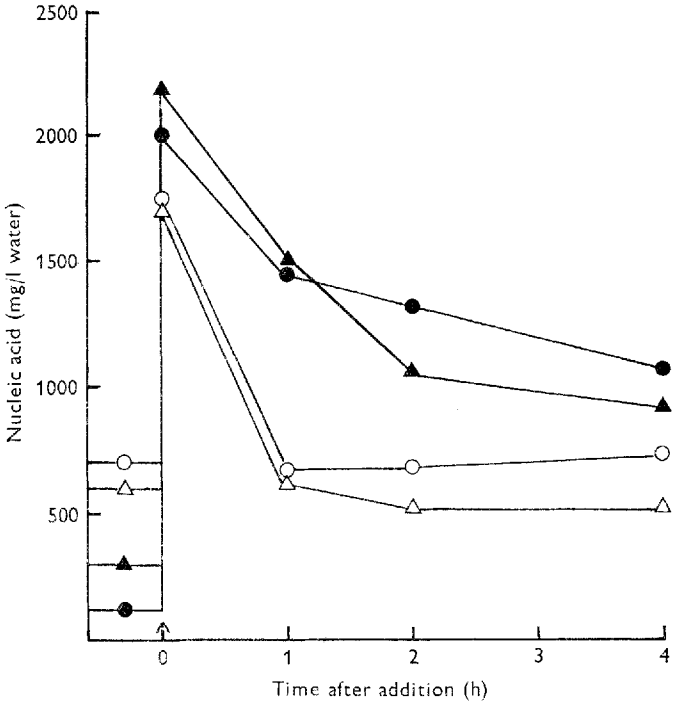

Fig. 2. Changes with time in concentrations of RNA (open symbols) and DNA (solid symbols) during anaerobic incubation, after adding the appropriate nucleic acid (55 mg yeast RNA or thymus DNA) to 24 g rumen contents from a cow (○, ●) or a sheep (△, ▲). Conditions of incubation are described on p. 332.

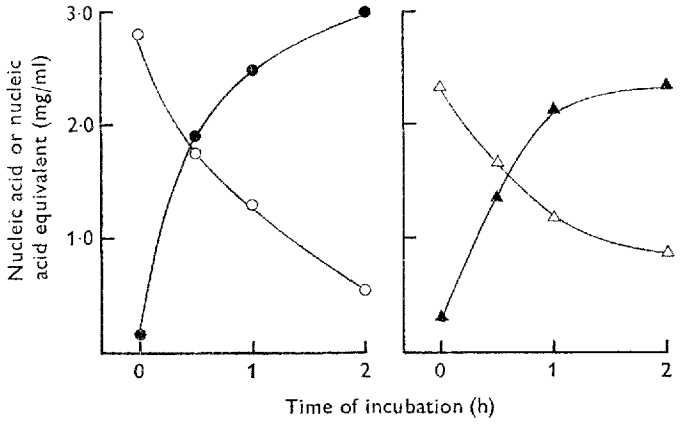

Fig. 3. Changes with time in concentrations of RNA (○), ultrafilterable material derived from RNA (●), DNA (△), and ultrafilterable material derived from DNA (▲) when 1 ml solutions, containing 55 mg/ml of the appropriate nucleic acid (yeast RNA or thymus DNA), were incubated with 24 g rumen contents obtained from a calf maintained on a diet of flaked maize and hay. Values for ultrafilterable materials were expressed as nucleic acid equivalents. The incubation procedure was as described on p. 332.

Table 4. *Derivatives produced from nucleic acids in vivo in the calf rumen and in vitro by incubation with rumen contents*

(In vitro experiments used either pure RNA or DNA or hay as nucleic acid source and incubation was carried out with samples of whole rumen contents or cell-free filtrates of rumen contents from calves receiving a diet of flaked maize and hay. In experiments in vivo, samples of pure RNA or DNA were added to the rumen of a calf at pasture. Detailed procedures are described on p. 332)

Treatment*	Time after adding substrate (h)	μmol/ml: Mono-† and oligo-nucleotides	Pyrimidine‡ nucleosides	Thymine	Uracil	Guanosine	Hypoxanthine	Xanthine
A. Incubation in vitro whole rumen fluid + RNA (5·96 μmol/ml)	1	2·40	0·21	0	0·34	0·21	0·40	0·34
	2	0·27	0·40	0	0·96	0	0·63	1·02
	4	0·01	0	0	1·52	0	0·44	1·34
B. Incubation in vitro whole rumen fluid + DNA (5·96 μmol/ml)	1	1·63	Trace	0·25	0·12	0	0·30	0·27
	2	1·70	Trace	0·55	0·26	0	0·26	0·50
	4	1·34	Trace	0·83	0·48	0	0·20	0·62
C. RNA addition – in vivo (approx. 6·60 μmol/ml)	0·75	1·37	0·16	0	0·20	0·07	0·09	0·16
	1·5	0·74	0·26	0	0·33	0·05	0·18	0·35
	4	0·01	0	0	0	0	0	0
D. DNA addition – in vivo (approx. 6·60 μmol/ml)	0·75	1·29	0·72	0·64	0·28	0·14	0·16	0·37
	1·5	0·94	0·39	0·44	0·10	0·08	0·10	0·16
	4	0·07	0·04	0·05	0	0	0	0
E. Whole rumen fluid + hay in vitro (3·60 μmol/ml)	2	0·25	0·37	0·28	0·15	0·19	0·26	0·16
	4	1·37	0·62	2·02 §		0·13	0·19	0·41
	6	1·51	0·69	2·21 §		0·10	0·11	0·51
F. Incubation in vitro cell-free rumen fluid + RNA (5·96 μmol/ml)	0·5	0·75	0	0	0	0	0	0
	2	0·85	0	0	0	0	0	0
G. Incubation in vitro cell-free rumen fluid + DNA (5·96 μmol/ml)	0·5	0·72	0	0	0	0	0	0
	2	0·85	0	0	0	0	0	0

* Nucleic acid concentrations are expressed as μmol base equivalents/ml.

† Areas are expressed as adenylic acid equivalents and corrected for u.v. absorbing material (0·16–0·30 μmol/ml) present in rumen contents.

‡ Total area measured and expressed as uridine equivalents.

§ Uracil and thymine peaks were not completely resolved. The total area of both peaks was measured and the mean relative value for uracil and thymine was used to calculate the concentration.

However, according to Davidson (1969) the extinction coefficient of a nucleic acid is significantly less than the sum of the extinction coefficients of its constituent nucleotides and Kunitz (1949–50) found a maximum increase in absorbance at 260 nm of 30% on enzymic degradation of DNA. From this, therefore, although the apparent mean percentage recoveries (± SEM) after 2 h were in four experiments with RNA 97 ± 8 and in four experiments with DNA 91 ± 8, there was in effect something like 35–40% loss of degradation products during the changes shown in Fig. 3. This loss was demonstrated more clearly in some samples when amounts of the individual degradation products formed were measured (see below and Table 4).

A small amount of ultrafilterable material which absorbed u.v. at 254 nm was always

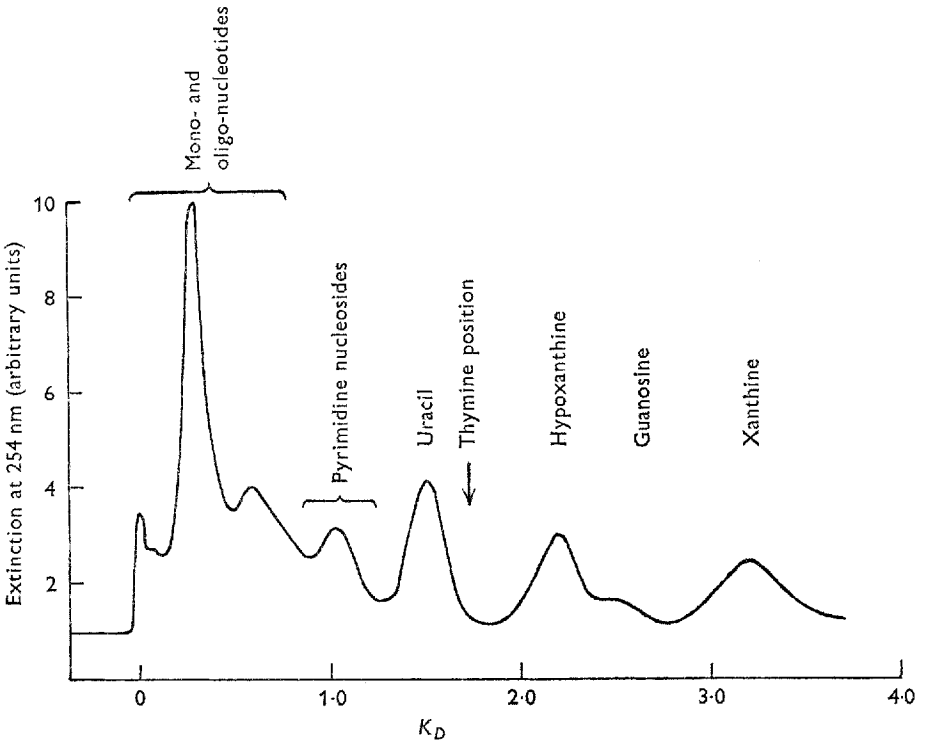

Fig. 4. Chromatogram on Sephadex G-10 of products of breakdown of nucleic acids in the rumen of the calf. A sample of rumen contents was obtained from a calf receiving flaked maize and hay, 90 min after infusion of 75 g yeast RNA into the rumen. The sample was ultrafiltered, 2 ml ultrafiltrate were applied to a Sephadex G-10 column (900 mm × 15 mm) and eluted with 0·01 M-ammonium acetate, pH 7·0. The chromatogram shown has been corrected for small amounts of u.v.-absorbing material (approximately K_D 0) present in an ultrafiltrate of fresh rumen contents.

present in samples of freshly collected rumen contents whether from calves at pasture or those receiving a diet of flaked maize and hay. Fractionation on G-10 showed that this material had a partition coefficient (K_D) value near to 0. Other experiments showed it to possess a u.v. absorption spectrum unlike that shown by nucleic acid derivatives. Substances which absorbed u.v. at 254 nm formed after nucleic acids were added to the rumen or in the course of incubating rumen fluid with nucleic acids were, on the other hand, found in peaks ranging from K_D 0 to K_D 3·5 and all showed u.v. spectra characteristic of nucleic acid derivatives. Several peaks were identified by their positions on the Sephadex chromatograms and by confirmatory experiments with the separated material using thin-layer chromatography. One such complete chromatogram is shown in Fig. 4 as an example. Peaks which were positively identified were xanthine, hypoxanthine, uracil, guanosine and (from DNA) thymine. The amounts of these substances were estimated from the areas under the peaks. Other peaks were identified as pyrimidine nucleosides but these were poorly separated from each other. Estimates of their amounts were obtained by using uridine as the reference material. Peaks appearing between K_D 0 and K_D 0·8 were less clearly

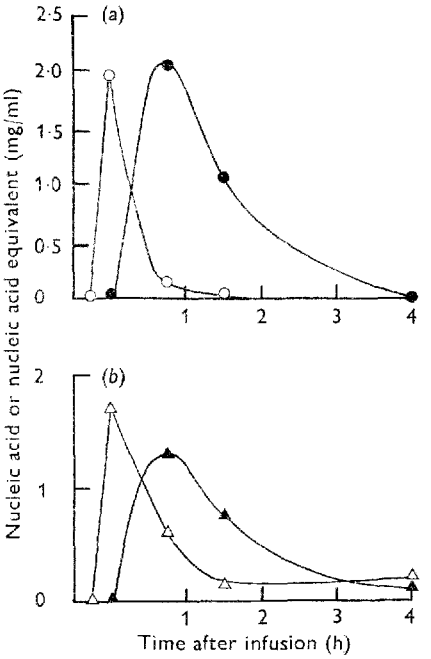

Fig. 5. Changes, with time, in RNA (○), ultrafilterable material derived from RNA (●), DNA (△) and ultrafilterable material derived from DNA (▲) after infusion of a solution containing 50 g yeast RNA or 50 g herring-sperm DNA into the rumen of a calf grazing pasture. The results are presented as increases in nucleic acid concentrations, or ultrafilterable materials (expressed as nucleic acid equivalents) over endogenous levels.

defined. Their positions suggested strongly that they consisted of mono- and oligo-nucleotides, and results were obtained by measuring the total area under the peaks and expressing these as adenylic acid equivalents. Values for typical experiments are shown in Table 4 (A and B) as examples; similar results were obtained with two samples of rumen contents from calves at pasture and two from calves receiving flaked maize and hay. The bases xanthine, hypoxanthine, uracil and (from DNA) thymine always accumulated in the reaction mixture, suggesting that they were relatively resistant to microbial degradation. No guanine, adenine or cytosine were detected. Furthermore, only small amounts of nucleosides were found, suggesting that their degradation to bases also occurred rapidly. These findings were confirmed by experiments in which whole rumen contents were incubated with certain nucleosides and purine and pyrimidine bases (McAllan & Smith, 1973).

Degradation of nucleic acids in vivo. Smith & McAllan (1970*a*) showed that pure nucleic acids, introduced into the rumen of calves, were rapidly broken down. In the present experiments samples of rumen contents were taken just before, and at intervals after, solutions (about 700 ml) containing 50–75 g yeast RNA, or herring-sperm DNA, plus 50–75 g PEG, were infused into the rumens of calves which were receiving a diet of flaked maize and hay (two experiments) or were grazing pasture (two experiments). The total rumen volumes of the calves used, estimated by dilution of added PEG, were 33–38 l. The samples were used for nucleic acid determinations but they

Table 5. *Ultrafilterable nucleic acid degradation products found in calf duodenal digesta*

(Samples of digesta were obtained from the proximal duodenum of a calf, receiving a diet of flaked maize and hay, at different times after infusion of a solution containing either 75 g yeast RNA or 75 g herring-sperm DNA into the rumen. Ultrafiltrates were prepared and 2 ml were applied to a Sephadex G-10 column (900 mm × 15 mm) and eluted as described on p. 333)

Treatment	Time of sampling (h) after infusion of RNA or DNA into the rumen	μmol/ml						
		Mono- and oligo-nucleo-tides*	Pyrimi-dine nucleo-sides*	Uracil	Thy-mine	Guano-sine	Hypo-xanthine	Xan-thine
RNA added to rumen	1	0·03	0·05	0·04	0	0	Trace	Trace
	2	0·03	0·17	0·26	0	Trace	0·13	0·32
	4	0·21	0·08	0·09	0	0	Trace	0·26
DNA added to rumen	1	0·32	Trace	Trace	0·13	0	Trace	Trace
	2	0·42	0·10	0·04	0·28	Trace	Trace	0·12
	4	0·34	0·16	0·04	0·09	0	0	0·13

* Calculated as described in Table 4.

were also ultrafiltered and the u.v. extinction at 260 nm of the ultrafiltrates was measured. Irrespective of diet, the percentage disappearances (mean ± SE) of the nucleic acids were similar to those found before; 100 ± 0 and 85 ± 9 for RNA and DNA respectively 1 h after their infusion.

The u.v. absorbance of the ultrafiltrates showed first a marked increase but by 4 h after introduction of the nucleic acids, in all experiments, had decreased nearly to the pre-infusion level. Typical examples are shown in Fig. 5. In four experiments the ultrafiltrates were fractionated on a column of Sephadex G-10. Peaks for substances showing K_D values of 1·0 and above, which were readily identifiable as pyrimidine nucleosides, uracil, hypoxanthine, xanthine, guanosine and (from DNA) thymine, were observed together with peaks with K_D values of about 0–0·8 which appeared to be due to mono- and oligo-nucleotides. These values are shown in Table 4(C and D) for one experiment with each of the nucleic acids. Similar results were obtained in experiments with calves at pasture (two experiments) and calves receiving flaked maize and hay (two experiments). Changes observed were generally similar to those found in the experiments in vitro, but the degradation of the nucleic acids (particularly DNA) and the nucleotides derived from them proceeded much more rapidly than in vitro. Further, the purine and pyrimidine bases, and particularly uracil and xanthine which were fairly resistant to degradation in vitro, disappeared completely after 4 h. In none of the experiments in vivo were more than trace amounts of added nucleic acids, or their derivatives, present in rumen fluid after this time.

Some of the nucleic acid degradation products formed left the rumen with the flow of digesta, passing into the omasum and more distal parts of the alimentary tract. Fractionation of samples of digesta obtained from the proximal duodenum of a calf receiving flaked maize and hay after RNA, in one experiment, and DNA in another

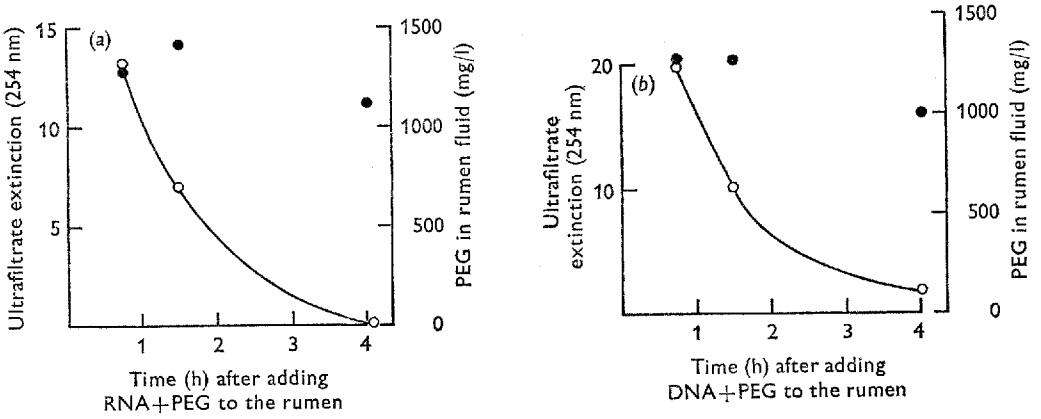

Fig. 6. Changes with time in ultrafilterable absorbing materials (○) and polyethylene glycol (PEG) (●) after infusion of solutions of (*a*) 75 g yeast RNA+75 g PEG or (*b*) 75 g herring-sperm DNA+ 75 g PEG into the rumen of a calf grazing pasture. Ultrafiltrates were prepared and examined, and PEG was estimated as described on p. 333.

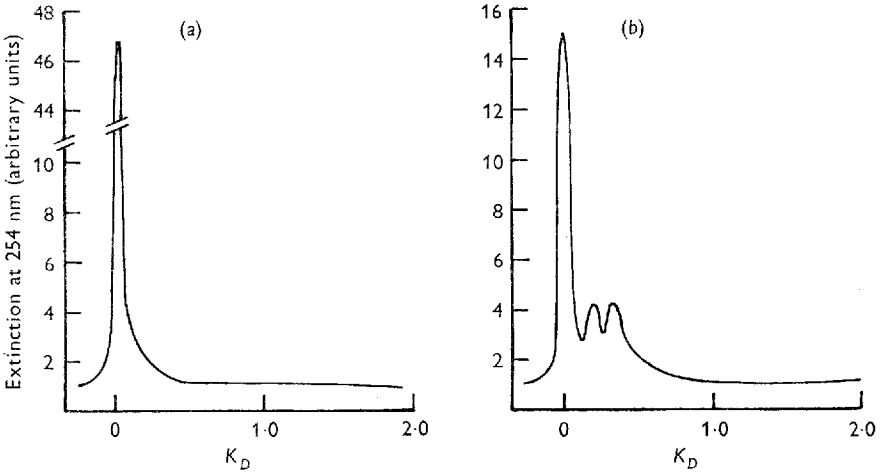

Fig. 7. Chromatogram on Sephadex G-10 of products of breakdown of nucleic acids produced by extracellular enzymes in rumen fluid. Samples of cell-free rumen fluid (24 g), prepared from rumen contents obtained from a calf receiving flaking maize and hay, were incubated, under the conditions described on p. 332, with 1 ml solutions of yeast RNA (55 mg/ml) or thymus DNA (55 mg/ml) for 2 h. Ultrafiltrates were prepared and 2 ml samples of these were applied to a Sephadex G-10 column and eluted with 0·01 M-ammonium acetate, pH 7·0. The chromatograms shown for (*a*) ultrafilterable material derived from DNA and (*b*) ultrafilterable material derived from RNA have been corrected for small amounts of u.v.-absorbing material measured after incubating cell-free rumen fluid for 2 h and for ultrafilterable u.v.-absorbing materials obtained from the relevant nucleic acid after incubation, at the same pH and concentration, for 2 h.

experiment, had been added to the rumen, showed that xanthine, hypoxanthine, uracil, pyrimidine nucleosides, mono- and oligo-nucleotide material and (from DNA) thymine, appeared transitorily at this site (Table 5). The amounts involved, calculated from any reasonable estimate of total digesta flow, were, however, very small in comparison to the amounts disappearing from the rumen. This was demonstrated direct

in other experiments in which both nucleic acids and PEG were infused into the rumen. The rates of disappearance of ultrafilterable u.v. (260 nm)—absorbing materials were much greater than the rates of disappearance of PEG (Fig. 6). It appeared, therefore, that another process was mainly responsible for the disappearance of the purine and pyrimidine bases from the rumen.

Degradation by 'cell-free' filtrate. Samples of cell-free rumen fluid were prepared from rumen contents obtained from calves receiving flaked maize and hay. These were incubated (24 g) with 1 ml solutions of yeast RNA (55 mg/ml) or thymus DNA (55 mg/ml) for varying times up to 2 h, as described on p. 332. Sephadex fractionation of ultrafiltrates, prepared from the reaction mixtures, showed that both RNA and DNA were converted into ultrafilterable oligonucleotides increasingly with time up to 2 h (Table 4, F and G), but no other degradation products were produced (Fig. 7).

Highly polymerized nucleic acids were degraded similarly on incubation with cell-free filtrate, but at a slower rate for both RNA and DNA. For example, after 2 h incubation the amounts of ultrafilterable material, expressed as adenylic acid equivalents, were 68 and 61%, for RNA and DNA respectively, of those obtained with non-highly polymerized nucleic acids.

Degradation of nucleic acids contained in hay. All experiments reported above were carried out with 'pure' nucleic acids. Under normal conditions, ruminants may ingest an appreciable proportion of their nitrogen intake as nucleic acids, particularly when their diets contain much hay (Smith & McAllan, 1970*a*). Incubation of a sample of ground hay with whole rumen contents from a calf receiving a diet of flaked maize and hay showed appreciable production of nucleic acid breakdown products (Table 4, E) and it appears that there was a fairly rapid release of nucleic acids from the cellular structure of the hay.

DISCUSSION

Any study of the metabolic changes occurring in the rumen is inevitably concerned with the net result of the action of a variety of different micro-organisms. The observed processes might be expected to change with variations in the composition of the microbial population resulting from different conditions. Such changes certainly may occur and may, for example, give rise to varying proportions of volatile fatty acids in the rumen (Sutton & Johnson, 1969). Nevertheless, the rumen processes as a whole are often reproducible under specified conditions even when, as with ureolysis, they appear to be carried out by organisms forming an insignificant part of the whole rumen microbial population (Cook, 1972). In fact, the total processes depend upon the combined action of a mixed microbial population adapted to the conditions obtaining at any one time. It may be difficult or impossible to simulate these processes by the use of single pure strains of rumen micro-organisms. In the present work no attempt has been made to examine individual micro-organisms; only the total effects of mixed populations developing under different conditions have been studied.

Rates of disappearance of nucleic acids incubated, in vitro, with rumen contents from cows, sheep or calves were all closely similar and, although the pattern of

degradation and formation of derivatives was examined only with calves, all the results were closely similar, irrespective of variation in diet. Thus, while it is necessary to bear in mind that, under special conditions, the rumen population and the total processes for which it is responsible may sometimes be different from those described in the present paper, it seems likely that the results presented here describe what happens under most 'normal' conditions.

The results obtained with cell-free rumen fluid suggest that at least part of the initial degradation of RNA and DNA in rumen fluid occurs through the action of nucleases which, although presumably of microbial origin, are extracellular. The complete absence of purine mononucleotides (the possible presence of small amounts of pyrimidine mononucleotides cannot be excluded because of their low K_D values (see Table 1 and Fig. 7)) and purine, and pyrimidine bases and nucleosides among the products formed by these extracellular enzymes suggests that both the ribonucleases and deoxyribonucleases present are predominantly endonucleases. Such enzymes have been reported as being produced extracellularly by bacteria from various sources other than the rumen (Kurnick, 1962; Barnard 1969; Voros, 1969).

Exonucleases and enzymes leading to further degradation of nucleotides and nucleosides have been found in a variety of bacteria but apparently only within or associated with the bacterial cell itself (Privat de Garilhe, 1967; Laskowski, 1967, 1968). In the present work, in vitro experiments with whole rumen fluid showed the rapid degradation of oligonucleotides to mononucleotides and simpler derivatives when bacterial cells were present. It seemed likely that an important degradative pathway included the dephosphorylation of one or more pyrimidine nucleotides and guanylic acid, since pyrimidine nucleosides and guanosine appeared transiently in the reaction mixture. Experiments with non-rumen micro-organisms have also suggested that guanosine is a product of nucleic acid degradation and is then commonly degraded by the pathway guanosine → guanine → xanthine (Colla, Craveri & Craveri, 1965). The absence of detectable amounts of adenosine during the degradation processes suggested that adenylic acid might have been degraded differently. There appears to be little information available on the usual pathway of adenylic acid degradation in micro-organisms, but in animal tissues it has been reported that deamination most probably occurs at the nucleoside level (Murray, Elliott & Atkinson, 1970). In the present experiments deamination of all purine or pyrimidine moieties bearing a side amino group occurred at some stage of the degradation process so that no adenine, guanine or cytosine were ever detected in rumen fluid after addition of nucleic acid, and the only bases found were xanthine, hypoxanthine, uracil and (from DNA only) thymine. Van der Horst (1965) reported that β-alanine and β-amino isobutyric acid were formed by the action of rumen bacteria on uracil and thymine respectively but, in the present work, products formed by further degradation of the bases in the in vitro experiments have not been characterized – these bases were rather resistant to degradation under conditions in vitro. This was particularly apparent for xanthine, uracil and thymine, the amounts of which steadily increased with incubation. It can, for example, be calculated that the xanthine recovered after incubation of RNA for 4 h (Table 4) accounted for about 89% of the guanine originally

present in the nucleic acid. This resistance to degradation shown by these bases was not apparent in the experiments in which nucleic acids were added to the rumens of calves in vivo. The reason for this is not clear. The bases formed in the rumen passed on, with the flow of digesta, into the more distal alimentary tract and were (cf. Table 5) detected in duodenal contents after either RNA or DNA had been added to the rumen. However, comparison with the flow of the unabsorbed and unmetabolized marker PEG from the rumen (Fig. 6) showed that the ultrafilterable products formed from the degradation of either RNA or DNA disappeared from the rumen much more rapidly than could be accounted for by digesta flow alone. Several possibilities for the fate of the bases in vivo can be suggested: (1) they are absorbed across or metabolized in the rumen wall; (2) they are metabolized more efficiently by the bacteria in vivo than in vitro; (3) they are taken up by ciliate protozoa in the rumen. The last is unlikely since our recent work (unpublished) has suggested that the rumens of calves kept under our conditions contained little or no protozoa. The other possibilities have not been resolved in the present work. The question is of more than academic interest since some ruminant foodstuffs, particularly hay, provide considerable intakes of nucleic acids (Smith & McAllan, 1970*a*) which may lead to appreciable amounts of purine and pyrimidine bases appearing in the rumen (Table 4). At present their fate is unknown.

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