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From the Department of Physiology, Veterinary College of Norway, Oslo.

FORMATION OF POLYAMINES IN THE RUMEN OF GOATS DURING GROWTH*

By

Knut Arnet Eliassen

ELIASSEN, KNUT ARNET: *Formation of polyamines in the rumen of goats during growth.* Acta vet. scand. 1982, *23*, 275—294. — Ornithine decarboxylase (E.C. 4.1.1.17) and S-adenosylmethionine decarboxylase (E.C. 4.1.1.50) and their products putrescine, spermidine and spermine were estimated in the rumen liquid from 3 groups of growing kids and 23 adult goats. Polyamines were also estimated in the feedstuff used. Marked differences in polyamine synthesis in rumen liquid were observed between the different groups of kids. Two groups of kids growing up together with adult goats had at an age of 2—4 months a peak of a few days duration in enzyme activity as well as in polyamine concentration. In these groups ornithine decarboxylase activity reached maximal values of 158±79 s (n=4) and 100 (66—117) (n=3) nmol[$^{14}CO_2$]/ml rumen liquid/h at an age of 120 and 77 days, respectively. The corresponding activity in rumen liquid from kids who were isolated from other animals was only about 1/10 of this value. By comparison ornithine decarboxylase activity in adult goats was 30.7±20 (n=43) nmol[$^{14}CO_2$]/ml/h.

In rumen liquid from kids grown up together with adults, concentrations of the polyamines reached maximum at about the same time as ornithine decarboxylase activity. The mean maximal concentration of putrescine in the 2 groups was about 350 and 500 nmol/ml, while the corresponding value for spermidine was about 200 nmol/ml in both groups.

Relatively constant and high concentration of polyamines were present in the feedstuff used. However, in growing kids the ruminal putrescine and spermidine concentration at times far exceeded those that could be accounted for by the estimated intake of polyamines by the food. The results therefore strongly indicate that polyamines are formed in considerable amounts in rumen content of kids during the phase of rapid growth. Results from a few experiments with calves also indicate that this may be true for cattle.

polyamines; putrescine; spermidine; spermine; ornithine-decarboxylase; rumen liquid.

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Although the physiological functions of polyamines are still largely unknown, their formation seems to be closely related to growth processes, and many studies have revealed changes in polyamine levels during growth in animals as well as in microorganisms and plants. Little information is, however, available regarding the formation of polyamines in the digestive tract of ruminants. In contrast to single-stomached animals, ruminants possess an enzyme in blood plasma which specifically degrades polyamines (*Hirsch* 1953, *Tabor et al.* 1954, *Blaschko & Hawes* 1959, *Blaschko & Bonney* 1962).

On the other hand, polyamines can influence the growth of some bacterial species in vitro (*Bachrach* 1973). By way of influence on the ruminal microflora, polyamines may therefore affect the growth of the forestomachs as well as the ruminant body as a whole.

The present investigation was initiated with the aim of obtaining information on polyamine formation in the digestive tract of ruminants during the transition from a functionally single-stomached animal to a functional ruminant. Most of the studies were done on goats, but some calves/cows were also included in the project.

MATERIALS AND METHODS

Animals and housing

Goats. All goats were of Norwegian dairy goat breed. Kids were obtained from the Agricultural University of Norway, Ås. Kids in Group I (2 males and 1 female) and Group II (3 males) stayed with their dams for a few days after birth and were transfered to our own barn at a live weight of 4.0 ± 0.4 kg. The animals were kept in indoor pens. The kids of Group III (6 males and 6 females) were kept at Ås together with other kids, during the last part of the experimental period in an outdoor pen.

For indoor pens, sawdust was used for bedding.

Due to increasing aggression the males in Group II were castrated at the age of 200 days.

Adult goats (27 females) also served as source for rumen fluid to be analyzed.

Cattle. Four calves and 3 cows kept under ordinary farm conditions were included in the study.

Feeding regime

Goat kids. The animals were fed at 8 a.m. and at 3^{30} p.m. Milk replacer was fed in a daily amount of 2×0.5 l (175 g/l) for weeks 1 through 5, 0.5 l throughout week 6. Hay, water, special concentrate for calves ("Kalvefôr") and a mineral mixture were given ad libitum from an age of 2, 3, 3, and 4 weeks respectively. At the age of 5 weeks the concentrate uptake was about 50 g per day, increasing gradually up to about 200 g by an age of 2—3 months. The goats at Ås were fed both hay and concentrate ad libitum. The concentrate intake for the Ås kids was after an age of 10 weeks about 3 times higher than for the kids in Groups I and II. The hay intake was correspondingly less.

Adult goats. These were fed hay and water ad libitum and 100 g special concentrate for cows ("Kufôr") per day.

Calves. During the first 33 days of life only milk replacer (175 g/l) and hay were used, after which the ration consisted of hay, water and concentrate ("Kalvefôr") ad libitum. The milk replacer consumption increased gradually to 5 l/day by the age of 2 weeks. After 5 weeks the amount of milk replacer fed was reduced with 1 l/week until termination in the 10th week.

Cows. The cows were kept on ordinary rations, hay and concentrate.

Feeds

The milk replacer ("Kip"®, kindly supplied by Peter Møller A/S, Oslo) contained 23 % digestible crude protein, minerals and vitamins, and represented 1.45 feed units per kg. "Kalvefôr" (calf feed) was a concentrate mixture in pelleted form, containing 14.5—16.5 % crude digestible protein and 0.94—0.98 feed units per kg. "Kufôr A" (cow feed A) was a concentrate mixture containing 14—16 % crude digestible protein and 0.93—0.95 feed units per kg. Both concentrate mixtures had vitamins added. They were obtained from Møllesentralen I/S, Oslo. The mineral mixture used was standard "Mineral Mixture" for ruminants and horses obtained from A/S Norsk Mineralnæring, Oslo.

Chemicals

DL-[1-^{14}C]-ornithine monohydrochloride was from the Radiochemical Centre, Amersham, England, S-[carboxyl-^{14}C]-adenosyl-L-methionine from New England Nuclear, Boston, Mass.,

U.S.A., and DL-carboxyl-[^{14}C]-arginine hydrochloride from Centre D'Etudes Nucléaries De Saclay, CEA, Ire Sorin, Gif-Sur-Yvette, France. Putrescine dihydrochloride, DL-ornithine hydrochloride, spermidine trihydrochloride, spermine tetrahydrochloride, DL-dithiothreitol, S-adenosyl-methionine chloride (grade II) pyridoxal-5′-phosphate, DL-proline and dansyl chloride were all obtained from Sigma Chemical Co., St. Louis, Miss., U.S.A.

Collection of rumen liquid

Rumen liquid was collected by means of a stomach tube and a vacuum pump. To avoid possible diurnal changes, all samples were collected between 12^{00} and 12^{30} p.m. Incubations for enzyme assays were started as soon as possible after sampling, usually within ½ h after collection. Samples collected at Ås were kept on ice for 1½ h before incubation. No differences in enzyme activity were found between samples incubated within ½ h and samples stored on ice for 2 h. HCl in quantities sufficient to bring pH down to about 2 was immediately added to samples to be used for polyamine determinations. Acidified samples were kept at —20° until analysed.

Determination of polyamines

The direct dansylation method of *Seiler & Wiechmann* (1967), modified for plant material by *Smith & Best* (1977), was used for the estimation of the polyamines. The dansyl-derivatives were dissolved in 100 µl water and extracted into 500 µl toluene. The toluene layer was evaporated and the residue resolved in 50—250 µl methanol before injection on the chromatograph. 1.6-diaminohexane was used as internal standard.

Thinlayer (TLC) and high performance liquid-chromatography (HPLC)

One dimentional TLC of the polyamine extracts was performed as described by *Smith* (1973) and *Smith & Best* (1977). TLC was used only in the first part of the experiments, later HPLC was used. There was a good correlation between the results obtained with HPLC and TLC, but HPLC was more sensitive, less variable and less time consuming. The HPLC procedure was, with some modifications, as described by *Seiler & Knödgen* (1978). Separation of dansyl-derivatives was achieved on a re-

versed phase column, μ Bondapak C 18, 3.9 × 300 mm (Waters Assoc.) using a gradient with 65 % methanol as solvent B and 100 % methanol as solvent A. The gradient was changed from 100 % of solvent B to 100 % of solvent A in 23 min at a flow-rate of 2 ml/min. All amines were eluted within 23 min. The column was allowed to re-equilibrate with solvent B for 5 min before next run. The dansyl-derivatives were quantified using a fluorescence detector. An excitation filter at 360 nm and a 460 nm cut off filter for the emitted light were used. It was possible to detect as little as 1--2 pmol polyamine per injection, but no less than 4 pmol could be determined with satisfactory degree of accuracy. In addition to the fluorescence detector, an U.V. detector (254 nm) was used to control the purity of the peaks, since the ratio between the fluorescence- and U.V. exitation most probably would change when impurities are present.

Enzyme determinations

Ornithine decarboxylase (E.C. 4.1.1.17) (ODC) and arginine decarboxylase (E.C. 4.1.1.19) (ADC). These 2 enzymes were assayed by measuring the rate of [$^{14}CO_2$] evolution from 0.2 μmol carboxyl labelled substrate, DL-[1-^{14}C]-ornithine and DL-[1-^{14}C]-arginine, as described by *Kobayashi et al.* (1971) and *Maudsley et al.* (1976). The specific activity of the 2 samples was 1.25 μCi/μmol. To a volume of 0.2 ml rumen liquid was added 1.8 ml of 0.1 M-phosphate buffer (pH = 5.4), 0.2 μM in pyridoxal phosphate, 2 μM in EDTA and 5 μM in dithiothreitol. The final pH of the reaction mixture was always within the optimum range for enzyme activity (5.6—6.5). Incubation time was 30 min.

S-adenosyl-L-methionine decarboxylase (E.C. 4.1.1.50) S-AMDC. This enzyme was assayed using a procedure similar to the one used for determination of ODC. As substrate was used 0.2 μmol S-adenosyl-L-[1-^{14}C]-methionine, specific activity 0.5 μCi/μmol. Phosphate buffer (0.1 M, pH 7.4) was used without putrescine and Mg^{++}.

Incubation in N_2-atmosphere, or in $N_2 + CO_2$-atmosphere resulted in enzyme activities not significantly different from incubations in air.

Enzyme activities are expressed as nmol [$^{14}CO_2$] produced per ml or g per h.

RESULTS

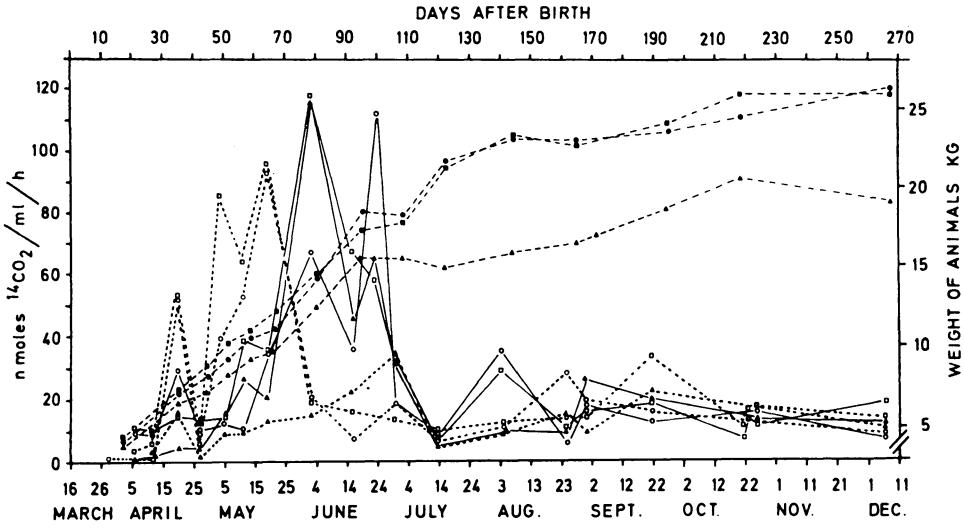
Enzymes studies

Rumen liquid from 4—5 month old goats kept on mixed ration was used in untreated form for these studies. Centrifugation at 20,000 × g for 30 min left little enzyme activity in the supernatant fluid, indicating binding to particles and/or microorganisms.

The pH optimum for ODC was found to be between 5.4—6.4. ADC showed a similar optimum range. S-AMDC showed very low activity at pH below 6.4, increasing slowly to maximum activity at pH 8.4. At pH 7.4 the activity was about 60 % of that at pH 8.4. However, since pH is rarely above 7.4 in normal rumen contents, incubations were made at this pH. S-AMDC was putrescine insensitive and was not stimulated by Mg^{++}. The pH in rumen contents was found to be 6.73 ± 0.4 (n = 73) and 6.43 ± 0.37 (n = 261) for Groups I and II, respectively.

Decarboxylases in rumen liquid

K i d s. The animals in Groups I and II which were studied from a very early point in time showed very low levels of ODC as well



F i g u r e 1. Ornithine decarboxylase (———) and S-adenosylmethionine decarboxylase (- - - - -) activity expressed as nmol [$^{14}CO_2$]/ml/h in rumen liquid from 3 kids in Group I and their growth curves (— —). □ kid No. 1, ○ kid No. 2 and △ kid No. 3. Filled symbols are used for the growth curves.

as S-AMDC activity in rumen liquid until the age of about 3 weeks (Figs. 1 and 2).

Group I: S-AMDC levels increased markedly in 2 of the 3 animals from the age of about 3 weeks, reaching maxima at about 60 days, followed by a rapid decline to lasting low levels. In the third animal no marked elevation was seen (Fig. 1). ODC-levels started increasing in all 3 animals at the age of about 50 days, declining rapidly at about 100 days of age and stabilizing at low levels thereafter (Fig. 1).

ADC activity was examined only at the age of 98, 108 and 212 days in these animals, and was found to be somewhat lower than the ODC activity.

Group II: The enzyme patterns recorded for these animals, which were studied the following year, deviated markedly from the patterns recorded for the Group I animals. Thus no marked elevation of the activity of any of the 3 enzymes were recorded at any point in time. Fig. 2 gives the results. The ratio ADC/ODC was about 2 the first month of life, but decreased to about 1 when the animals passed the age of 3 months.

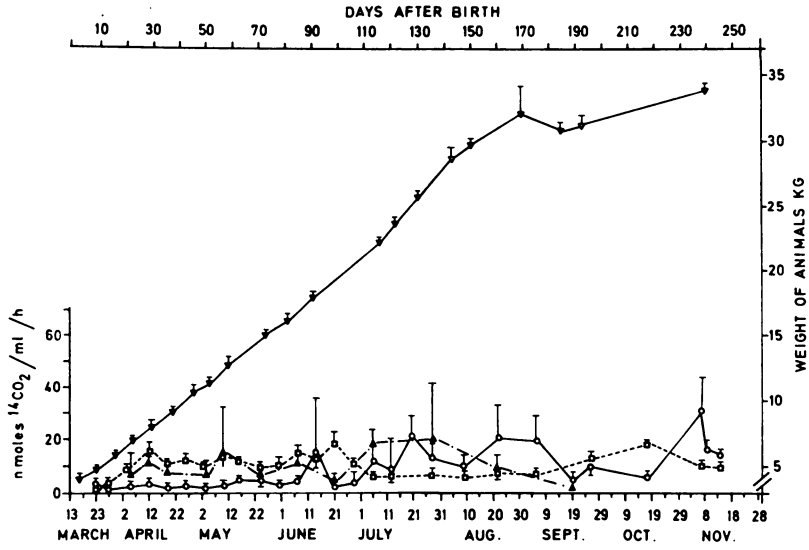


Figure 2. Ornithine decarboxylase (○——○), arginine decarboxylase (△—·—△) and S-adenosylmethionine decarboxylase (□---□) activity, expressed as nmol [$^{14}CO_2$]/ml/h, in rumen liquid from kids in Group II and their growth curve (▽——▽). The plotted values represent means derived from 3 kids with s.e.m. indicated.

Group III: Due to the markedly different patterns in enzyme activities seen between the animals in Groups I and II, a third group of animals, including 12 kids, was studied. As shown in Fig. 3, high levels of ODC activity were found in all animals in the age range 91—123 days, with decrease to moderate levels in the age range 140—190 days. The youngest animals (born between 2nd and 6th April) reached maximal ODC activity at an earlier age than those born earlier in the year.

ADC activity in Group III animals was examined only twice, at an age of about 4 and 5 months and was 15.7 ± 9.5 s (n = 6)

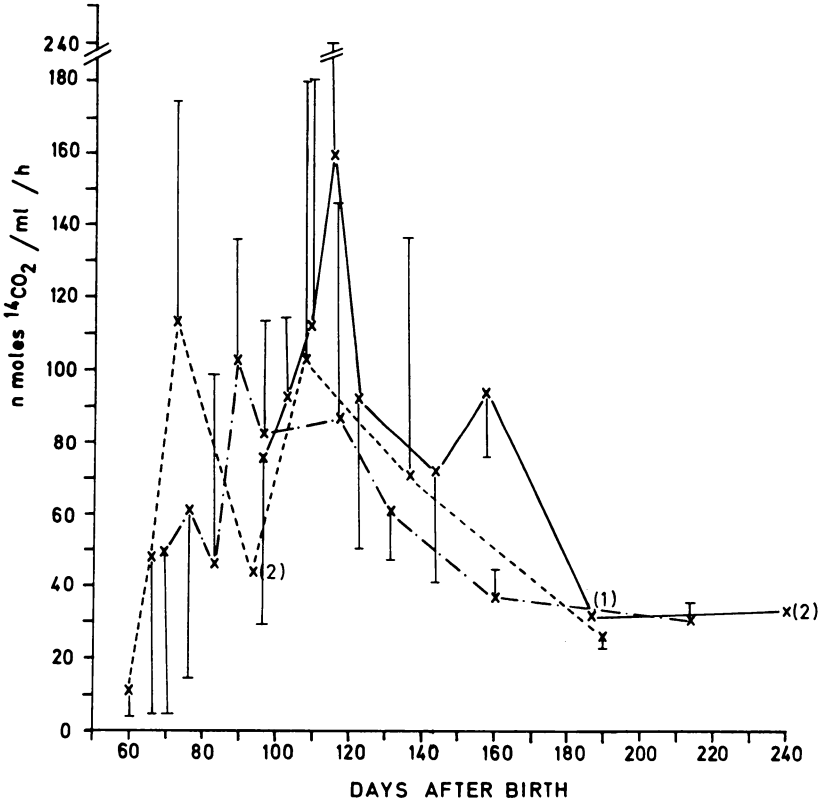


Figure 3. Ornithine decarboxylase activity, expressed as nmol [$^{14}CO_2$]/ml/h, in rumen liquid in Group III.

———— kids born between February 13. and 19.
—·—·—·— kids born between March 1. and 19.
- - - - - - kids born between April 2. and 6.

Values are means of duplicate determinations in each of 4 kids. Vertical lines indicate the s of the 4 means, if not otherwise stated on the figure.

and 9.5 ± 3.4 s (n = 6) nmol/h, respectively, the ratio ADC/ODC being 1:4.

S-AMDC activity was not measured in Group III.

A d u l t g o a t s. ODC activity in rumen liquid from adult goats showed considerable variation between animals and also from time to time for the same animals without showing any systematic relationship with the age. During the period June to October the average value was 29.7 ± 20.5 s nmol/ml/h (n = 43, 27 animals). In the period when the kids in Group III showed the highest ODC activity in rumen liquid, the corresponding activities for dams were, with only 1 exception, much lower (data not shown).

C a l v e s a n d c o w s. Table 1 gives the values for ODC and S-AMDC activities in rumen liquid from 4 calves and 3 cows. For calves the ODC activity was at least 8 times higher at the age of 33 or 47 days than at 73 days of age, at which time the values had decreased to approximate cow levels. The values for S-AMDC were less variable and much lower than for ODC.

T a b l e 1. Ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (S-AMDC) in rumen liquid from cattle expressed as nmol $[^{14}CO_2]$/ml/h.

Animal	Calves						Animal	Cows	
No.	33 days		47 days		73 days		No.	2—4 years	
	ODC	S-AMDC	ODC	S-AMDC	ODC	S-AMDC		ODC	S-AMDC
1	76	30	44	8.2	7.5	10.8	5	5.9	5.8
2	4	6.8	75	10.7	9.4	10.2	5	1.95	2.4
3	47	2.5	88	45	8.2	5.6	6	12.9	5.3
4	518	7.3	242	14	8.7	10.2	6	4.5	4.4
							7	4.3	7.3
Mean±s	161±240	11.6±12.4	112±88	19.5±17.2	8.5±0.8	9.2±4.2		5.9±4.2	5.0±1.8

Polyamines in rumen liquid of goats

The levels of the polyamines in rumen liquid of goats during growth showed considerable variation.

Group I: Due to technical difficulties, polyamine determination on rumen liquid in this group of animals was not started until the animals were about 3 months old. Highest polyamine levels were found at an age of about 100 days (Fig. 4), corresponding

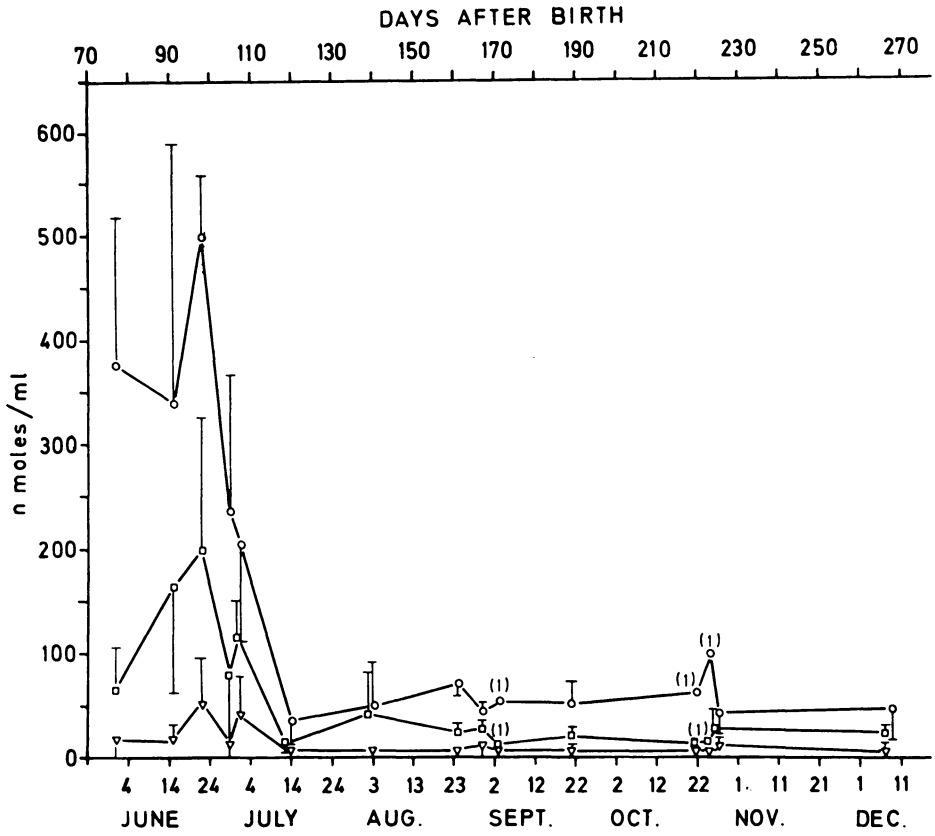


Figure 4. Polyamine concentration in rumen liquid samples from kids in Group I: ○ putrescine; □ spermidine; ▽ spermine. Values are means of duplicate determinations in each of 3 kids. Vertical lines indicate maximum deviation from the mean for 3 kids.

roughly with the peak levels of ODC activity (Fig. 1). From the age of about 120 days the levels of all 3 polyamines were invariably low.

Group II: The polyamine levels were very low during the first 20 days of life, after which a rapid increase in the levels of putrescine and spermidine was observed. The highest putrescine concentration appeared about 30—45 days after birth, with a subsequent decrease. The spermidine concentration varied considerably and no systematic relationship between age and spermidine concentration was observed. From the age of about 150 days putrescine and spermidine levels stabilized at approximately

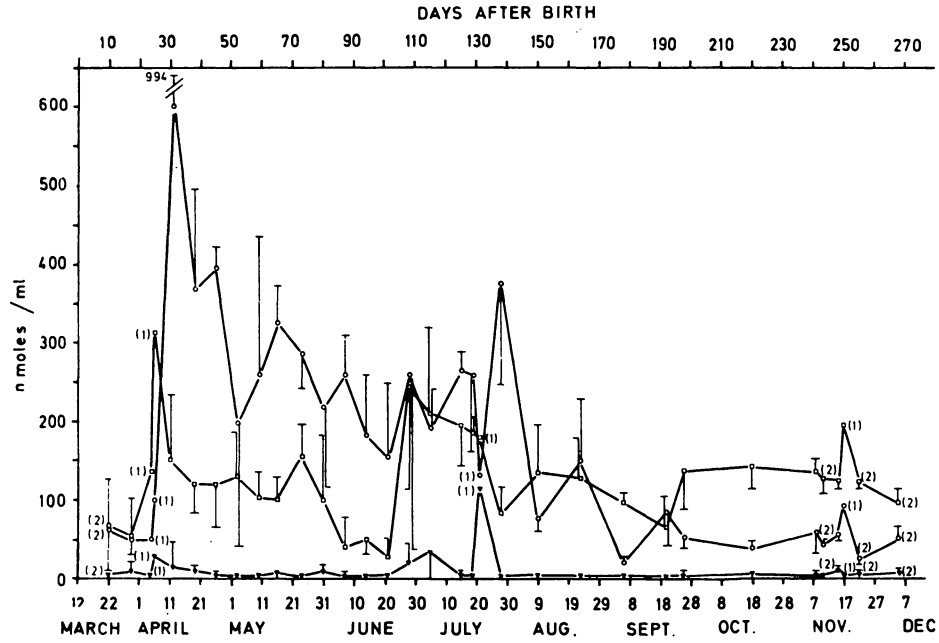


Figure 5. Polyamine concentration in rumen liquid samples from kids in Group II: ○ putrescine; □ spermidine; △ spermine. Values are means of single determinations from 3 kids. Vertical lines indicate maximum deviation from the mean for 3.

the same and 3 times the levels as seen for Group I, respectively. Spermine levels were low throughout the period studied (Fig. 5).

Group III: As shown in Fig. 6, the polyamine levels found from the age of about 100 days were of the same order of magnitude as the values found in Group I for the corresponding ages. Changes in polyamine concentrations varied roughly with those of the ODC activity.

Polyamines in feed

Since the occurrence of polyamines in rumen contents might result from uptake of polyamine containing feed, it was necessary to analyze the feeds used for these substances. As shown in Table 2 considerable amounts of putrescine as well as spermidine and spermine were recorded in all feeds used.

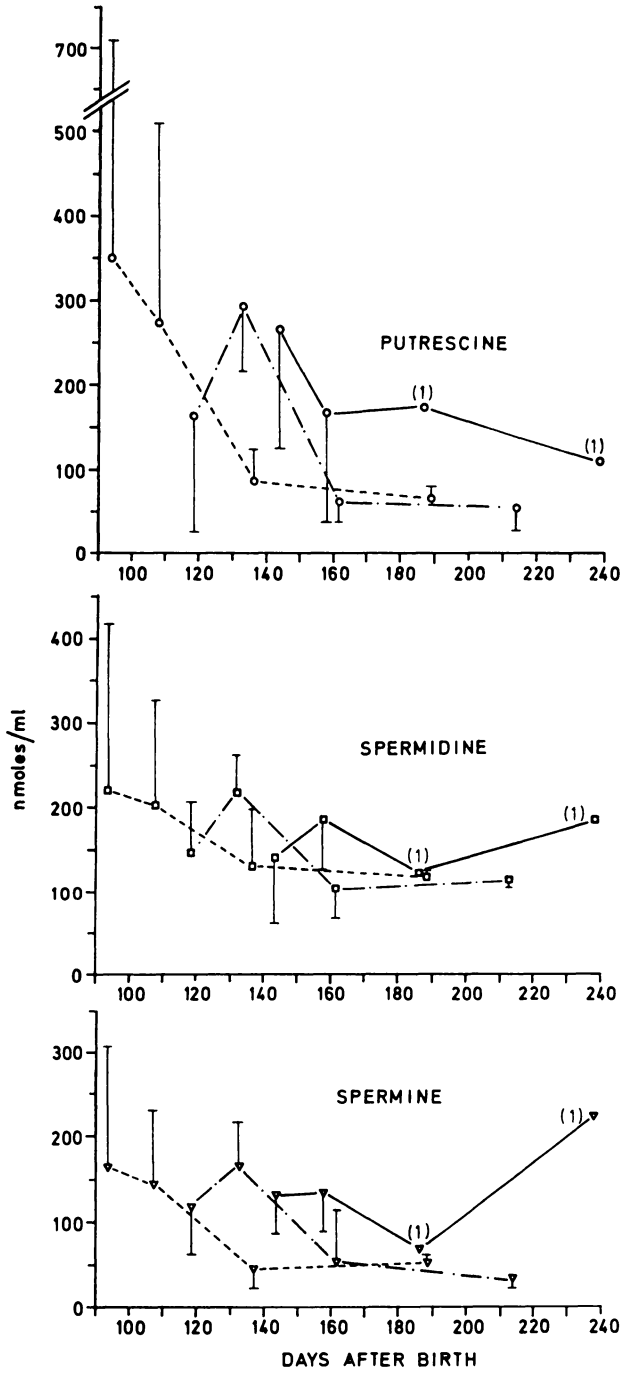


Figure 6. Polyamine concentration in rumen liquid samples from kids in Group III.

———— kids born between February 13.—19.
· — · — · — · kids born between March 1.—19.
– – – – – – – – kids born between April 2.—6.

Values are means of single determinations from 4 kids. Vertical lines indicate s.

Table 2. Polyamine content in the feed.

Food	Putrescine nmol/g±s (n)	Spermidine nmol/g±s (n)	Spermine nmol/g±s (n)
Milk substitute, Kip®	93±53 (5)	151±46 (5)	45±19 (5)
Hay	250±78 (12)	56±22 (12)	49±31 (12)
Concentrates, Calf feed	395±59 (4)	147±21 (4)	65±14 (4)
„ , pelleted Calf feed	346±55 (7)	157±37 (7)	69±20 (7)
„ , Cow feed A	246±45 (10)	206±24 (10)	59±13 (10)

DISCUSSION

Methods

Although up to 99 % of the total ruminal flora is present in the rumen liquid, the metabolic activity of rumen liquid in vitro may not reflect the processes taking place in vivo. Thus histamine is degraded to a considerable extent in the rumen in vivo but not when incubated with rumen contents in vitro (*Kay & Sjaastad* 1974). Recently microorganisms adhering to the ruminal epithelium have been reported to be different from strains found in the liquid (*Cheng & Costerton* 1980). On the other hand changes or differences in activity levels in rumen liquid supposedly will reflect real events and may thus give additional information.

The decarboxylase assays were based on CO_2 trapping. The CO_2 requirement of the rumen microorganisms might thus represent a source of error. However, when the CO_2 trapping agent was added after incubation, the results were not significantly different from those obtained when CO_2 trapping took place during incubation.

The TLC and HPLC methods used for determination of polyamines in rumen liquid and the feeds gave satisfactory results for spermidine and spermine. Complete separation of putrescine and 1.3-diaminopropane was not achieved, but the latter compound seemed to be present in so small amounts (if any) that influence on the putrescine values is most unlikely.

Sources of ruminal polyamines

Formation of polyamines by rumen microbes has been assumed (*Blaschko & Hawers* 1959), but evidence for this hypothesis seems to be lacking. Our findings show that polyamine

forming enzymes, as well as polyamines like putrescine and spermidine at times were present in very high concentrations in rumen liquid, indicating formation in the rumen. However, as the feed analyses showed, polyamines were present in high concentrations also in the feeds used. The contribution of the feed to the total contents of polyamines in rumen liquid was therefore estimated. Since neither the food consumption nor the total volume of the rumen contents were accurately measured, this estimation must be based on some assumptions, namely:

1) that the rumen contents of kids at 1 week of age represent about 1 % of the body weight, increasing to about 10 % at the age of 3 months;

2) that the feed consumption was approximately as given in Table 3.

Table 3. Estimated feed intake (g/day) for kids in Group I and II at different ages.

	Group I		Group II			
Age in days	98	119	21	35	120	151
Milk replacer (Kip®)	0	0	175	175	0	0
Hay	600	750	50	50	500	1000
Concentrates	200	200	0	50	200	200

Based on these assumptions, the maximum values for polyamines in rumen liquid that might stem from the feed and the concentration present in the rumen liquid of kids at various ages are given in Table 4. The concentration of spermine in rumen liquid constantly seemed to be lower than corresponding to the estimated intake. For putrescine and spermidine, at times much higher concentrations are found in the rumen liquid than could be accounted for by the estimated intake, this being most marked for putrescine.

From these observations it seems difficult to draw clearcut conclusions. However, the rumen represents a dynamic system, in which factors like formation, degradation, absorptive processes and flow rates for digesta will influence the net values measured. However, the observation that the changes in polyamine concentrations in rumen liquid from most of the kids were roughly related to the changes in ODC activity (Figs. 1, 3,

Table 4. Estimated contribution from feed of polyamines in ruminal contens, compared to values determined in the contents.

		Group I		Group II			
	Age in days	98	119	21	35	120	151
Putrescine	contributed						
nmol/ml±s	by the feed	126	119	144	124	116	109
	determined	504± 53	33±19	48±15	602±347	260±45	77±12
Spermidine	contributed						
nmol/ml±s	by the feed	35	32	145	93	33	10
	determined	217±101	16± 8	56±43	152± 80	39±33	137±56
Spermine	contributed						
nmol/ml±s	by the feed	23	22	51	39	21	23
	determined	55± 38	~0	7±12	15± 26	2± 7	6± 3

4 and 6), together with the calculations presented, strongly suggest that polyamine formation indeed takes place in the rumen. The low values found for ruminal spermine, compared with the concentrations of this polyamine in the feeds used, point to considerable degradation or absorption of this substance.

Polyamine forming enzymes and their sources

Correspondence between changes in enzyme levels and polyamine levels in rumen liquid was not always observed. Discrepancies might be explained by a) participation of an enzyme in addition to ODC and S-AMDC in the formation of polyamines, and/or b) variation in degradation rates for ruminal polyamines. With respect to a), ADC is a candidate, since it has been shown to be of significance in polyamine formation in bacteria and in plants. In the present investigation the ADC/ODC ratio (results not presented) was highest for animals showing the poorest correlation between polyamine and ODC levels (Group II). However, there was no significant correlation between the ADC activities and the polyamine concentrations (Figs. 2 and 4). ADC seemed to be quantitatively more important than ODC in the polyamine formation in the first 2 months of life. Thereafter the ADC/ODC ratio decreased with age.

As regards b), no information is available as to degradation rates for polyamines in rumen, but histamine, a diamine, is degraded to a considerable extent in this compartment (*Kay & Sjaastad* 1974). The fact that the increase in S-AMDC-activity

preceded the increase in the activity of ODC, which catalyzes an earlier step in the polyamine synthesis, does not exclude the possibility that the 2 enzymes may be formed in the same strains of bacteria. However, the relative availability of the substrates, partly originating from the feed, may vary with time, and thus lead to variations in induction of the 2 enzymes in question.

If polyamine formation takes place in the rumen, the question whether the enzymes involved are of bacterial or protozoan origin cannot be answered on the basis of the present investigations. A preliminary bacteriological examination did not reveal any systematic relationship between bacterial counts and polyamine levels. Further, the total number of bacteria was found to be very high even before any significant increase in polyamine forming enzymes was observed (*Almlid et al.*, unpublished data). This observation does not, however, exclude a connection between certain strains of bacteria and polyamine formation, since the relative number of bacteria of different strains may change dramatically from time to time.

Protozoa were not counted. According to *Hungate* (1966), a pH of 6.6, which was common in the samples of the rumen liquid examined, favors development of protozoa which may play a role in polyamine formation and/or degradation. This remains to be shown.

Possible reasons for differences in enzyme levels between animals

Within the kid groups the differences in enzyme levels between animals were moderate. However, when the groups were compared, marked differences were observed (Figs. 1, 2 and 3). The animals showing the lowest levels of enzyme activity (Group II) were kept in individual pens, isolated from adult animals for the first 4 months of life. On the other hand, peaks in rumen liquid ODC activity coincided in time for animals of varying ages kept together in the same pen. Thus differing possibilities with respect to the establishment of a normal ruminal flora may explain at least some of the large differences in enzyme activity profiles observed between the groups of animals. According to *Hungate* (1966), young animals separated from faunated animals shortly after birth, may require artificial inoculation to establish a normally faunated rumen.

Enzyme and polyamine levels in relation to growth

No systematic relationship between enzyme and polyamine levels in rumen liquid and development of rumen and growth rate was observed (Figs. 1 and 2). All kids in Groups I and III, and also all calves, showed temporary elevation of polyamines as well as of polyamine forming enzymes, compared to later levels in the same animals and to other adult animals. The explanation may be that when young ruminants are reared in the presence of adult ruminants, the establishment of the ruminal flora is a gradual process, including changes in the relative contribution of various bacterial strains to the total microbial metabolism.

The concentrations of cadaverine, histamine and tyramine were also determined along with the polyamines, but they showed no systematic pattern of variation. This difference between polyamines and other amines might be an indication of a physiological role of the polyamines in the development of the forestomach.

Spermine oxidase, an enzyme degrading polyamines, is present in blood plasma of ruminants. According to *Blaschko et al.* (1959, 1962) and *Ueyama et al.* (1965), the increase in the level of this enzyme in plasma occurs concommitantly with the development of the rumen. This corresponds to the time of elevated levels of polyamines and polyamine forming enzyme activity in rumen liquid of young animals reared in the presence of adults. In preliminary investigations we have shown that the growth of some bacterial strains isolated from rumen liquid is influenced by polyamines added to the medium (*Almlid et al.*, unpublished data). Thus polyamines may indirectly influence the growth of ruminants by influencing the balance between various strains of rumen bacteria. At the same time the increase in plasma spermine oxidase protects the growing ruminant against possible deleterious effects of polyamines that might be absorbed from the gastro-intestinal tract. However, it is noteworthy that the high activity of the polyamine forming enzyme, as well as the high polyamine concentrations in the rumen contents from kids reared in the presence of adults, are transient while blood plasma spermine oxidase remains at a high level.

It would seem that under normal conditions, polyamines are formed in increased amounts in rumen contents during the phase of rapid growth (Fig. 1), the formation being, at least partly,

mediated by bacterial enzymes, as suggested by *Blaschko & Bonney* (1962). The contribution of polyamines from feeds to the total content of polyamines in the rumen should not be neglected, but since the changes in polyamine levels in rumen liquid during growth phase can hardly be related to changes in food polyamine uptake, de novo formation in the rumen must be considered a normal process.

The physiological functions of polyamines during the development of the rumen and at later stages remain undefined.

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SAMMENDRAG

Dannelse av polyaminer i vomma hos geit i vekst.

Ornithin dekarboksylase (E.C. 4.1.1.17) og S-adenosylmetionin dekarboksylase (E.C. 4.1.1.50) og deres produkter putrescin, spermidin og spermin ble målt i vomsaft fra 3 grupper av geitekje og 23 voksne geiter. Videre ble polyaminkonsentrasjonen i fôret bestemt.

Store forskjeller i polyamindannelsen i vomsaft fra de forskjellige gruppene av kje ble funnet. Kjeene i 2 grupper som vokste opp sammen med voksne dyr hadde da de var 2 til 4 måneder gamle, en forbigående topp i aktiviteten av de polyamindannende enzymer så vel som i polyaminkonsentrasjonen. I disse 2 gruppene fant en maksimale ornitindekarboksylase aktiviteter på 158 ± 79 s (n = 4) og 100 (66—117) (n = 3) nmol[$^{14}CO_2$]/ml vomsaft/time da dyrene var henholdsvis 120 og 77 dager gamle. Tilsvarende aktivitet i vomsaft fra kje som hadde vært isolert fra andre dyr under oppveksten var bare omkring 1/10 av denne verdien. Ornitindekarboksylase aktiviteten i vomsaft fra voksne geiter var til sammenlikning 30 ± 20 s (n = 43) nmol[$^{14}CO_2$]/ml/time.

I vomsaft fra kje som vokste opp sammen med voksne geiter nådde polyaminkonsentrasjonen maksimale verdier omtrent samtidig med at ornitindekarboksylase aktiviteten var maksimal. Den gjennomsnittlige maksimale konsentrasjonen av putrescin i vomsaft fra de 2 gruppene var omkring 350 og 500 nmol/ml. De tilsvarende verdier for spermidin var like for begge gruppene, omkring 200 nmol/ml.

I fôret fantes relativ høy og konstant konsentrasjon av polyaminer. Hos kjeene oversteg til visse tider konsentrasjonen av putrescin og spermidin i vomsaft tydelig de mengder som var beregnet å kunne være tilført med fôret. Resultatene viser at polyaminene i betydelige mengder dannes i vomsaft hos kje under hurtig vekst.

Resultatene fra noen forsøk med kalver tyder på at det tilsvarende er tilfelle også for storfe.

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Reprints may be requested from: Knut Eliassen, the Department of Physiology, Veterinary College of Norway, P.O. Box 8146, Dep., Oslo 1, Norway.