The effect on digestion in the rumen of a gradual increase in the content of fatty acids in the diet of sheep

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1. Two sheep were given cubed rations in which the fatty acid content was increased by about 12 g/day at the end of each week, until the daily intake became 74 g. 2. The apparent digestibility of the crude lipid increased with the increased intake, but there was no tendency for the digestibility of the fatty acids to increase or decrease regularly. 3. Comparison of faecal losses during the final periods, when a maximum amount of lipid was given, with the losses during the initial control periods showed that the excretion of dry matter, crude lipid and cellulose increased. The excretion of crude protein was unchanged and that of lipid- and cellulose-free dry matter decreased. There was a reduction in the production of methane of 17 kcal/100 kcal of additional fatty acids in the diet. 4. The concentration of the C_{18} unsaturated acids in the rumen liquor reached a maximum during the 1st week on the diet containing the maximum amount of added fatty acids. The shape of the curves was consistent with rapid hydrogenation of linolenic acid. The concentration of stearic acid in the rumen liquor reached a maximum when the dietary acids increased to only 50% of their maximum value.

It was shown by Czerkawski, Blaxter & Wainman (1966*a*) that infusion of unsaturated fatty acids into the rumen of sheep led to a marked lowering in the production of methane. The same authors (1966*b*) found later that the production of methane could be lowered also by incorporating the unsaturated, and, to a somewhat smaller extent, the saturated long-chain fatty acids in the diet. Most of these experiments did not last longer than 4 weeks, and no attempt was made to increase the fatty acid content of the diet gradually.

The turnover of microbial population in the rumen is relatively large, and it was judged unlikely, at least on the basis of previous work (Czerkawski *et al.* 1966*a*) that it would take longer than 3 weeks on a given regimen to establish an equilibrium. It was noticed by Czerkawski *et al.* (1966*a*) that methane production began to fall at once when 40–80 g of linseed oil fatty acids were infused daily into the rumen of sheep, and that it reached a steady low value after 3–8 days of infusion, the length of time varying with the amount infused. After infusion was stopped it took somewhat longer for the methane production to return to the normal value.

The object of the experiment now described was to increase the fatty acid content of the diet in suitable steps, and to examine the effect on digestion in the rumen. It was felt that 7 days on any one transition diet would be ample, because each contemplated increment would be less than 20 g/day, an amount which had previously been found to give only a very small reduction in methane production and which resulted in a steady new value of daily methane production after only 2–3 days.

EXPERIMENTAL

Animals. Two castrated male sheep with permanent rumen cannulas were used and kept in metabolism cages throughout except for 2-day periods at the beginning and end of the experiment, when they were placed in respiration chambers (Wainman & Blaxter, 1958) for methane production to be measured.

Rations. The basal concentrate diet was in the form of cubes which had the following composition in kg: barley meal 40·0; oat husk 20·0; oatmeal 20·0; sugar-beet pulp 5·0; dry grass meal 72·6; NaCl 1·5; $CaHPO_4$ 0·75; MgO 0·15; total 160·0 kg. Of this mixture 80 kg were cubed to give the control diet. The remaining 80 kg were mixed with 8·0 kg linseed oil fatty acids (British Drug Houses Ltd, Poole, Dorset; linoleic acid so-called), and then made into 'fatty acid' cubes.

The sheep were given 900 g control ration or 990 g fatty acid cubes daily in two equal meals. They were also given 150 g dried grass with the evening meal only. These constituted regimens A and F respectively; the intermediate rations (B–E), were prepared by mixing the control cubes and the fatty acid cubes in suitable proportions. The crude lipid and fatty acid composition of all the rations is summarized in Table 1.

Table 1. *Lipid content of the rations*

Diet	Nominal amount of oil added (g/day)	Crude lipid content as analysed		Fatty acid content as analysed	
		g/day*	Increment	g/day*	Increment
A	0	40·2	—	11·7	—
B	18	58·5	18·3	24·1	12·4
C	36	76·7	36·5	36·5	24·8
D	54	94·9	54·7	49·0	37·3
E	72	113·1	72·9	61·4	49·7
F	90	131·4	91·2	73·9	62·2†

* Includes the lipids derived from the cube rations and from the dry-grass supplement.

† The linseed oil fatty acid perparation contained 68·9 % fatty acids, of which 63·5 % was linolenic acid. This proportion of fatty acids would give normally 61·9 g/day increment in ration F compared with the 62·2 g found.

Plan of experiment. The sheep were given control ration A for 3 weeks. They then received ration B each day throughout the 4th week, ration C in the 5th, ration D in the 6th, ration E in the 7th and ration F in the 8th, 9th and 10th weeks.

During the last 4 days of the period on each diet the faeces excreted by the sheep were collected and bulked for analysis. On the last day of the various periods two samples of rumen contents (about 50 ml each) were withdrawn, one 2 h after the morning feed and one just before the evening feed. The samples were withdrawn by means of a probe made of perspex. A tube closed at one end was fitted into a shorter tube (outer diam. 1·6 cm). Longitudinal slits 15 cm long and whose width was equivalent to $\frac{1}{3}$ of the circumference were cut in each tube. The low end of the slits was about 2 cm from the closed end of the tubes. The probe was introduced into the rumen in the closed position (slits at 180° to each other). It was opened by turning the outer tube (slits coincident), gentle suction was applied at the top of the inner tube, and the

probe was closed by turning the outer tube. The probe was withdrawn and the contents, representative of about 15 cm of the vertical cross-section of the rumen, were transferred to a flask. This procedure gave between 10 and 15 ml of contents and was repeated four times during each sampling to give about 50 ml in all. The morning and evening samples from each sheep were weighed and equal amounts were pooled and analysed.

Analyses. The total lipids in the rumen contents were extracted by the method of Folch, Lees & Stanley (1957); the faecal lipids were extracted in the same way, but the material was previously treated with hot 2N-HCl to convert the soaps into fatty acids. The methyl esters of the total fatty acids in the various samples were prepared and isolated by the method of Kates (1964). They were subsequently analysed on the Pye argon gas–liquid chromatograph with 15% polyethylene glycol adipate, and sometimes Apiezon L, as the stationary phase on chromosorb W at 197°. The *cis*- and *trans*-isomers of octadecenoic acids were sufficiently resolved on Apiezon L columns to give a fair estimate of their relative distribution. Sometimes it was necessary to separate these components on thin-layer plates of silicic acid impregnated with 10% (w/v) silver nitrate before gas–liquid chromotography. No attempt was made to determine the position of the double bond in the octadecenoic acids. The feed lipids were analysed in the same way.

Cellulose was estimated by the method of Crampton & Maynard (1938), and other routine analyses, such as those for nitrogen, carbon and calorific values, were as described by Armstrong, Blaxter & Graham (1957).

There was usually good agreement between the fatty acid contents of the samples obtained from the two sheep. For instance, the average deviation from the means of the results for the major component fatty acids (palmitic, stearic, monoenoic C_{18}, linoleic) from the rumen and the faeces of the two sheep was usually less than 5%. The difference between the duplicates was greater with the minor components. The results reported in this paper are expressed as means for the two sheep.

RESULTS

Intake and excretion of lipids

Crude lipid and total fatty acids. It is shown in Table 1 that the intake of crude lipid during the control period was 40 g/day and that during the final period on ration F it reached 131 g/day. The excretion of crude lipid was 23 g/day during the control period and increased to about 41 g/day during the period on ration F, i.e. the amount excreted was doubled when the intake was increased three times. The intake of fatty acids was increased gradually from 12 g/day to a maximum value of 74 g/day, and this resulted in an increase in excretion of fatty acids from 1·6 g/day to about 8 g/day.

These results, expressed as apparent digestibilities, are summarized in Table 2. The digestibility of the crude lipid increased significantly during the gradual increase in the lipid content of the diet. On the other hand, the digestibility of the non-fatty acid portion of crude lipid varied from about 24·5% on ration A to almost 41% during the 3rd week on ration F, most of the intermediate values being about 31%. The

increase in digestibility of crude lipid can be explained by the inclusion of increasing proportions of the more digestible fatty acids.

Excretion of individual fatty acids. It was convenient to show the relation between the excretion of the individual acids and their daily intake by calculating the ratio of excretion to intake. Clearly, this ratio subtracted from unity and multiplied by 100 would give the corresponding apparent digestibility. These ratios are plotted in Fig. 1. It should be noted that small values such as were found with the polyunsaturated fatty acids mean that only between 0·5 and 5 % of the ingested acid was excreted in the faeces, a large proportion of this being of endogenous origin. The ratio was greater than 1 with stearic acid (Fig. 1*a*) which means that the amounts of stearic acid excreted were greater than the amounts ingested. This was to be expected, because stearic acid is the end-product of hydrogenation of the unsaturated C_{18} acids. The

Table 2. *Apparent digestibility* (%) *of the lipids by two sheep*

Diet	Crude lipid	Fatty acids	Non-fatty acid lipid
A	42·1	86·8	24·5
B	55·9	91·9	30·8
C	56·0	85·0	31·5
D	—	—	—
E	60·6	74·7	32·0
F (1st week)	56·6	87·5	19·0
F (2nd week)	64·0	89·4	31·5
F (3rd week)	68·6	90·3	40·6

extent of excretion of palmitic acid is also shown in Fig. 1*a*. The ratios of oleic, linoleic and linolenic acids are plotted in Fig. 1*b*. The ratios dropped consistently at first when the dietary acids were increased, but they then increased, particularly with oleic acid. The ratios dropped again when the diet richest in fat (ration F) was given, indicating that the animals were becoming adapted to the new conditions.

The ability to deal with an influx of additional fatty acids is particularly noticeable with linolenic acid. When the intake of this acid was 4 g/day (diet A), about 2 % of the acid was excreted in the faeces; when the intake was increased to 43 g/day, only 0·5 % of it was excreted. In other words the excretion of linolenic acid was only doubled when the intake was increased ten times.

It was of interest to inquire into the nature of the C_{18} monoenoic acids in faeces. The dietary monoenoic acid was almost wholly in the *cis*-form. During the control period (ration A) only 17 % of the C_{18} monoenoic acid in the faeces was in the *trans*-form; the remainder occurred as oleic acid, the major portion of which was probably of endogenous origin. When the monoenoic acids in the faeces from the final period (the 3rd week of ration F) were analysed, it was found that 87 % had the same retention volume on Apiezon L as elaidic acid. This is consistent with previous findings (Czerkawski & Blaxter, 1965) that a *trans*-monoenoic acid might be an intermediate in the hydrogenation of polyenoic acids in the rumen.

The bacterial fatty acids, which include the branched-chain C_{15} to C_{18} acids, and the acids with odd numbers of carbon atoms (C_{13} to C_{19}), were found in the rumen

and in the faeces but occurred in negligible amounts in the diet. They would be formed mainly in the rumen, and their excretion increased from 160 mg/day during the control period on ration A to about 400 mg/day on ration E. The excretion then began to fall and finally became 260 mg/day during the 3rd week of ration F. The increase was due mainly to an increase in acids with an odd number of carbon atoms, particularly C_{19}.

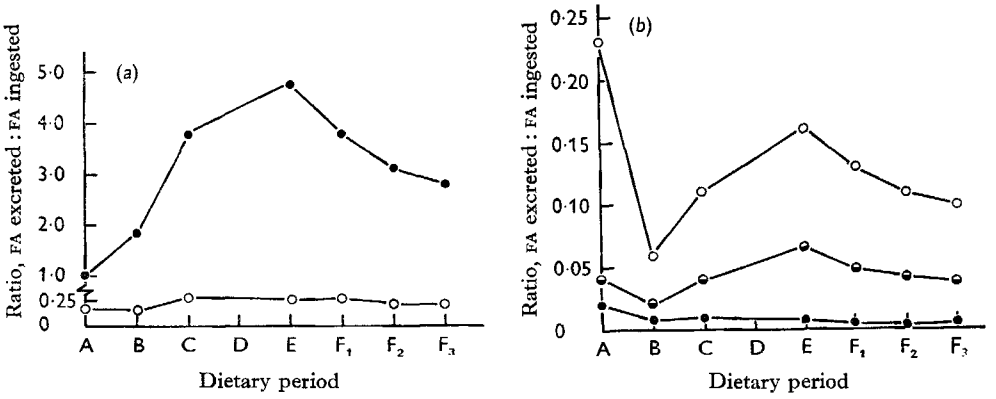

Fig. 1. Variation of the ratio of the amount of fatty acid excreted in the faeces to the amount of fatty acid (FA) given in the diet of two sheep. A is the last week on the control diet, B–E are the weeks on the diets with increasing fatty acid content (see p. 834), and F_1, F_2, F_3 are the 1st, 2nd and 3rd weeks on the diet F which had the maximum fatty acid content. (*a*) ●—●, stearic acid; ○—○, palmitic acid; (*b*) ○—○, octadecenoic acid; ◒—◒, linoleic acid; ●—●, linolenic acid.

Table 3. *Intake and faecal excretion of various components by two sheep*

Dietary period	Dry matter	Crude protein	Crude lipid	Cellulose	Lipid- and cellulose-free dry matter
			Daily intake (g)		
A–F	788–882	101	40–131	267	483
			Daily excretion (g)		
A	308·2	34·0	23·3	118·2	166·7
B	312·0	34·4	25·8	120·6	165·0
C	328·0	35·4	33·7	130·0	164·3
D	311·5	32·5	(39·0)*	121·4	(140·3)*
E	314·2	30·8	44·5	124·7	145·0
F (1st week)	356·7	35·3	57·1	146·9	152·7
F (2nd week)	335·5	34·6	47·4	142·8	145·3
F (3rd week)	332·1	33·2	41·2	140·5	150·4
Change†	+23·9	−0·8	+17·9	+22·3	−16·3

* Estimates by interpolation.
† Excretion during the 3rd week on diet F − excretion on diet A.

Intake and excretion of non-lipid components and loss of energy as methane

Table 3 shows the daily intakes and faecal excretions of dry matter, crude protein, crude lipid and cellulose, and of the lipid- and cellulose-free dry matter.

The increased loss of dry matter in faeces (23·9 g) was partly due to increased faecal lipid content (17·9 g). There was virtually no change in the loss of crude protein. The

excretion of cellulose was increased (22·3 g); this was more than was found previously during infusion of similar fatty acids. The last column of Table 3 shows that the excretion of the lipid- and cellulose-free dry matter was decreased (16·3 g/day).

The mean daily production of methane fell from 35·5 l. during the control period to 23·5 l. during the last week on ration F, when the sheep were receiving some 74 g fatty acids/day. Thus there was a reduction of more than 30% in the production of methane, whereas the digestion of cellulose was decreased by only about 14%. Expressed in a different way, this means that 100 kcal of additional linseed oil fatty acids produced a decrease in the formation of methane equivalent to 17 kcal. Previous experiments have shown that continuous infusion of these acids gave a decrease of 16 kcal methane/100 kcal fatty acids, and incorporation into concentrate cubes that were given twice daily but without gradual adaptation gave a drop of 28 kcal methane/100 kcal fatty acids (Czerkawski *et al.* 1966*b*).

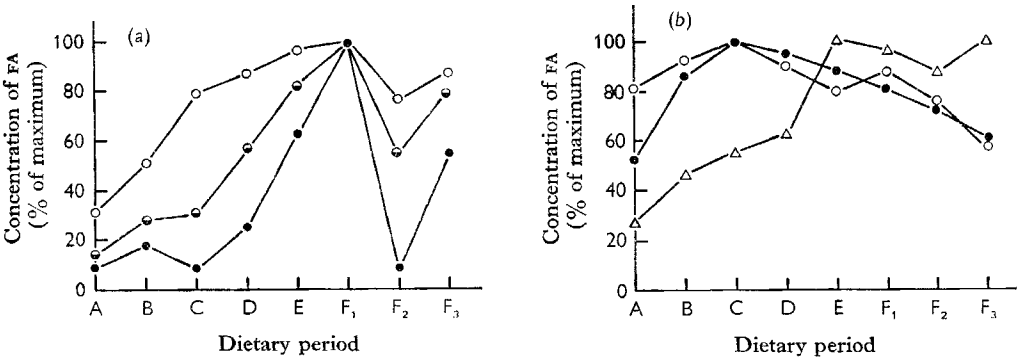

Fig. 2. Concentration of fatty acids (FA) in the rumen of two sheep, expressed as a percentage of the maximum value for any given acid. A represents the last week of the control period, B–E are the weeks on the diets with increasing fatty acid content (see p. 834), and F_1, F_2, F_3 are the 1st, 2nd and 3rd weeks on diet F, which had the maximum fatty acid content. (*a*) ○—○, octadecenoic acid; ◒—◒, linoleic acid; ●—●, linolenic acid; (*b*) ○—○, palmitic acid; ●—●, stearic acid; △—△, bacterial fatty acids (sum of branched-chain and odd carbon number fatty acids).

Fatty acids in the rumen

It has already been shown in previous sections that the addition of fatty acids to the diet produced little change in the overall digestibility of the acids, and that the ruminal flora is still apparently capable of hydrogenating the unsaturated acids.

The more immediate fate of the dietary fatty acids was investigated by determining their concentration in the rumen. Table 4 gives the amounts of individual acids consumed per day, and the concentration in the rumen at the end of each period. The concentrations, expressed as a percentage of the maximum value for any given acid, are plotted in Fig. 2.

The curves for the three unsaturated C_{18} acids (Fig. 2*a*) are similar; they all show that a maximum value occurred at the end of the 1st week on ration F, i.e. when the dietary fatty acids had reached their maximum value. According to these curves the results for linolenic and linoleic acids were more closely related to each other than to the results for the monoenoic C_{18} acids. The rapidity with which the concentration of

the three acids approached the maximum value and began to fall depended very much on the degree of unsaturation of the acids. It was twice as great for linoleic and four times as great for linolenic as for monoenoic acid. This probably reflects the rapidity with which these acids are hydrogenated. Linolenic acid is hydrogenated rapidly (Shorland, Weenink, Johns & McDonald, 1957; Lough & Garton, 1958) so it would not be expected to accumulate until the influx reached a certain saturation point.

Table 4. *Intakes of individual fatty acids by the two sheep and their concentration in the rumen*

Dietary period	16:0*	18:0	18:1	18:2	18:3	Others†
	Daily intake (g)					
A	1·6	0·3	2·3	2·9	3·7	0·1
B	2·2	0·5	4·3	4·7	11·6	0·1
C	2·8	0·7	6·3	6·5	19·4	0·1
D	3·4	1·0	8·4	8·3	27·3	0·0
E	4·0	1·2	10·4	10·1	35·2	0·0
F (3 weeks)	4·5	1·4	12·4	11·9	43·1	0·0
	Concentration in the rumen contents (g/5 l.)					
A	2·7	7·2	2·5	0·5	0·2	0·6
B	3·1	11·6	3·7	1·0	0·4	1·0
C	3·3	13·5	5·7	1·1	0·2	1·2
D	3·0	12·8	6·3	2·1	0·6	1·3
E	2·6	11·9	7·1	3·0	1·4	2·1
F (1st week)	2·9	11·0	7·2	3·6	2·3	2·0
F (2nd week)	2·5	9·9	5·6	2·0	0·2	1·8
F (3rd week)	1·9	7·7	6·4	2·9	1·3	2·2

* The first figure refers to the number of carbon atoms in the chain; the figure after the colon refers to the number of double bonds.

† Sum of branched-chain and odd carbon number fatty acids, possibly of bacterial origin.

Table 5. *Composition of octadecenoic acids in the rumen of two sheep*

Dietary period	Concentration in the rumen (g/5 l.)	
	Oleic acid	Elaidic acid*
A (control)	1·0 (40)†	1·5 (60)
E	1·9 (27)	5·3 (73)
F (3rd week)	0·8 (14)	5·5 (86)

* Identified tentatively as described by Czerkawski & Blaxter (1965).

† Figures in parentheses are percentages of the total octadecenoic acid.

The curves for the concentrations of the two unsaturated acids (Fig. 2*b*) differ considerably from those of the unsaturated acids. Both have a maximum value at the end of period C, i.e. when the amount of fatty acid in the diet had been raised to only about half its maximum value. The concentration of these acids in the rumen then began to fall slowly at first (up to the 1st week of ration F) and then more rapidly.

The curve relating the concentration of fatty acids of bacterial origin to the dietary period differs from all the other curves. The concentration reached the maximum value at the end of period E and then remained approximately constant.

There was an accumulation of *trans*-octadecenoic acid in the rumen when the amount of linolenic acid in the diet was increased (Table 5). The proportion of oleic acid in the rumen during the control period was smaller (40% of monoenoic acids) than in the faeces during the same period (83%). During the final experimental period (the 3rd week on ration F) the proportion of the *trans*-acid in the rumen was nearly the same as in the faeces (86 and 87% of octadecenoic acids).

DISCUSSION

The digestibility of stearic acid incorporated in the diet might be smaller than that of stearic acid newly formed in the rumen. When small amounts of stearic acid (20–39 g/day) were incorporated into diets of sheep, between 80 and 90% of this acid was apparently digested (Czerkawski, unpublished results). In one experiment the digestibility dropped to about 60% when the amount of stearic acid was increased to 50–60 g/day. The digestibility of stearic acid appears to be low in omnivorous animals, where the value rarely exceeds 50% (Hilditch & Williams, 1964), except perhaps when it is part of a mixed triglyceride. Stearic acid has a high melting point (69°) and even its sodium salt is only sparingly soluble in water. Thus it would tend to remain in a relatively coarse form and it might not be readily accessible to micro-organisms.

During the present experiment, when the sheep were on the 'fatty acid' diet, the intake of stearic acid was 1·4 g/day and the intake of unsaturated C_{18} acids was 67·4 g/day. If the latter acids were fully hydrogenated in the rumen and if all the acids passed without loss into the abomasum, the abomasal intake would be 68·8 g of stearic acid/day. Ulyatt, Czerkawski & Blaxter (1966) have shown that 85% of unsaturated fatty acids are hydrogenated in the rumen, and if one assumes that these results can be applied here then the total amount of stearic acid leaving the rumen might be 58·5 g/day. During the last week on ration F the sheep excreted 7·2 g of fatty acids per day and 56% of this was stearic acid (4·0 g). Therefore the apparent digestibility of the combined dietary and newly formed stearic acid was 93%. This calculation ignores a possible further hydrogenation in the caecum (Ward, Scott & Dawson, 1964).

There is evidence of lipolytic activity in the rumen (Garton, Hobson & Lough, 1958; Garton, Lough & Vioque, 1961), most of the glycerides of the diet being rapidly converted into free fatty acids. The hydrogenation of unsaturated fatty acids presumably takes place within the micro-organisms or on their surface (Williams, Gutierrez & Davis, 1963). Thus the newly formed stearic acid would be intimately associated with the organisms, probably being absorbed on the surface, and so be readily available for absorption lower down in the gut.

When in earlier work the linseed oil fatty acids were infused into the rumens of sheep, there was a reduction in the production of methane without any marked reduction in cellulolysis (Czerkawski *et al.* 1966*b*). This was not achieved in the present work mainly because of the choice of basal diet, which was suitable for ease and reproducibility of sampling, but was poor with regard to digestibility of cellulose. However, methanogenesis and cellulolysis were affected to different extents, methano-

genesis being reduced by 30% and cellulolysis by 14%. The digestion of crude protein was virtually unaltered and there was an improvement in the apparent digestibility of the non-lipid, non-cellulose dry matter of the ration. The ability of the rumen microflora to hydrogenate unsaturated fatty acids as their intake increased was not impaired.

Hydrogenation of unsaturated fatty acids in the rumen is at present one of the best known but least understood processes of rumen metabolism. It may simply be the outcome of the prevailing reducing conditions in the rumen, or it may be that there is some underlying biochemical requirement for such a process in the metabolism of certain rumen micro-organisms. Little is known about possible steps in the conversion of linolenic into stearic acid in the rumen. It appears that there is a tendency for the *trans*-octadecenoic acid, which does not occur in normal diets, to accumulate in the rumen (Czerkawski & Blaxter, 1965), and that linolenic acid is hydrogenated faster than either oleic or linoleic (Hoflund, Holmberg & Sellmann, 1956; Lough & Garton, 1958; Shorland *et al.* 1957). Many studies of steps in hydrogenation of unsaturated C_{18} acids have been conducted in vitro (Polan, McNeill & Tove, 1964; Ward *et al.* 1964; Kepler, Hirons, NcNeill & Tove, 1965), and a general scheme for hydrogenation of linolenic and possibly linoleic and oleic acids has emerged recently (Wilde & Dawson, 1966). It is still not certain whether such a scheme is applicable to conditions in vivo, such as reported here. The present results are not inconsistent with such a scheme except perhaps with respect to conjugation. Many in vitro studies indicate that the conjugated isomers of linoleic and linolenic acids might be intermediates in their biohydrogenation (Kepler, Hirons, McNeill & Tove, 1966) and yet there was no evidence of any significant bond migration during the present studies in vivo.

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