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FEEDING IN SHEEP DURING INTRAPORTAL INFUSIONS OF SHORT-CHAIN FATTY ACIDS AND THE EFFECT OF LIVER DENERVATION

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SUMMARY

1. Castrated male sheep were prepared with cannulae in the hepatic portal vein and jugular vein through which infusions lasting for 3 hr were made. Animals had free access to a pelleted feed the weight of which was continuously recorded so that feeding behaviour could be studied.

2. Infusion into the portal vein of a mixture of salts of short-chain fatty acids (acetate, propionate, butyrate: 55, 30, 15) caused a dose-dependent depression in food intake, feeding stopping completely with 4·0 m-mole/min of the mixture. Jugular infusion depressed intake slightly, compared with controls.

3. Separate infusions of salts of the three acids showed that the effect of the mixture was due almost entirely to its propionate content; 1·2 m-mole/min of propionate into the portal vein almost completely prevented feeding (39 g eaten per 3 hr) compared with jugular infusion at the same rate (210 g) or no infusion (205 g).

4. Surgical sectioning of the hepatic nerve plexus around the wall of the hepatic artery was attempted. Of seven animals which recovered normal food intake, three continued to eat during portal vein infusions of propionate at 1·2 m-mole/min; these sheep were subsequently shown to have been at least 95% denervated. One animal was 50% denervated and ate normally during some infusions but not others. In the remaining three, feeding was suppressed by portal vein infusion of propionate, and these were less than 75% denervated.

5. It was concluded that there are receptors in the liver which are sensitive to propionate and which have afferent fibres in the hepatic plexus.

INTRODUCTION

The ruminant is able to adjust its food intake to meet its energy requirements under many circumstances and it is likely that it uses products of digestion as satiety signals (see review by Baile & Forbes, 1974). The major energy-yielding substrates absorbed from the ruminant digestive tract are short-chain fatty acids, in particular acetate, propionate and butyrate, so attention has been focused on these as being

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likely to be sensed within the body to terminate feeding. Although the digestion of the insoluble part of the feed is slow, there is rapid fermentation of the soluble fraction of the feed which could yield sufficient absorption of short-chain fatty acids to cause the end of a meal (Forbes, 1980). Several workers, in particular Baile and his colleagues, have studied the effects on feeding of infusions of metabolites into the rumen or vascular system (see Baile & Forbes, 1974). Acetate depresses food intake more when infused into the rumen than when infused into carotid artery, jugular vein or hepatic portal vein, and it has been proposed that there are receptors sensitive to acetate in the rumen wall (Baile & Mayer, 1968); Harding & Leek (1972) demonstrated the existence of chemoreceptors in the rumen wall which were sensitive to change in pH, but not specifically to acetate. Propionate infusions were most effective in depressing intake when infused into the ruminal vein and on this evidence Baile (1971) postulated propionate receptors in the wall of that vein. It would be more logical, however, to expect propionate to be sensed in the liver because a large proportion of propionate passing in the hepatic vein is taken up by the liver and converted to glucose (Bergman, 1975). There is increasing evidence from non-ruminant species that the state of hepatic glucose uptake or release is transmitted to the brain via the autonomic nervous system to control feeding behaviour (Niijima, 1969; Novin & Vanderweele, 1977; Russek, 1970). Butyrate in rumen fluid is largely converted to β-OH butyrate as it is absorbed through the rumen wall and is quantitatively less important than either acetate or propionate.

The study reported here re-investigated the relative importance of these short-chain fatty acids as depressors of food intake when infused into the hepatic portal vein and explored the route whereby the particular effect of propionate on the liver is relayed to the central nervous system.

METHODS

Castrated male sheep of mixed breeding, aged between 6 and 18 months and weighing 42–57 kg were prepared with catheters (o.d. 2·0 mm polyvinyl, NT2, Portex Ltd., Hythe) in the hepatic portal vein via a mesenteric vein by a method similar to that of Harrison (1969). Anaesthesia was induced by I.V. pentobarbitone (Sagatal, May & Baker, Dagenham, approximately 15 mg/kg) and maintained by a halothane and oxygen mixture (Fluothane, I.C.I., Macclesfield) administered via a cuffed endotracheal tube. The animals were fed *ad libitum* on a 0·5 barley:0·5 dried grass pelleted feed (Hi D, Lincolnshire Farm Feeds, Grimsby) for 2 weeks until 2 days before surgery and experiments began when they had recovered at least their pre-operative level of intake; this usually took about 1 week.

Jugular vein catheters (NT2 or nylon, Portex) were introduced percutaneously at least 24 hr before jugular infusions. All catheters were flushed daily with 100 u./ml. heparinized saline.

Denervation of the liver was performed as follows. The animal was fasted for at least 24 hr, anaesthetized as detailed above and laid on its left side. An incision was made on the right flank parallel to and close behind the last rib, extending from just below the lateral process of the 1st lumbar vertebra for approximately 200 mm. The anterior edge of the incision was retracted rostrally by a large Kelly retractor together with the last rib. The retractor was tensioned by hooking onto an adjustable frame mounted around the anterior thorax. The head end of the operating table was raised and the kidney bridge was elevated in order to expose the portal vein more conveniently. The liver was held away from this vein by a retractor padded with gauze. The sympathetic nerves (hepatic plexus) innervating the liver run along the hepatic artery and this was confirmed histologically in the sheep.

In early attempts at denervation the portal vein was cleared of connective tissue but this was abandoned after some fatal haemorrhages during dissection. In all operations the hepatic artery was palpated and dissected free of the portal vein and common bile duct for at least 20 mm, starting about 50 mm from the porta hepatis. This piece of artery was then carefully cleared of surrounding connective and nervous tissue. The portal vein was then cannulated as described above.

At the end of each denervation experiment the sheep was killed by severing the carotid arteries under deep anaesthesia and the operated area was dissected out and prepared for histological examination using standard methods. The embedded tissue was sectioned (15 μm) and every fortieth section was stained with haemotoxylin and eosin and examined (see Plate 1).

Feeding behaviour was monitored continuously by suspending the feed bucket from a metal beam on which strain gauges (Texcel, St Albans) were bonded. These strain gauges, one on the top side and the other beneath the beam, formed two arms of a Wheatstone Bridge circuit, balanced by two resistances of similar value (120 Ω). Power to the bridge was provided by a constant 9 V supply and the output signal was fed to a paper chart recorder (Speedomax G, Leeds and Northrop, Tylseley, Birmingham).

Infusions were made at 1·0 ml./min by a peristaltic pump (Minipuls II, Gilson Medical Electronics, France) and lasted for 3 hr starting 30 min after the daily offering of fresh food. pH was adjusted to 7·3 before every infusion. Infusions of isotonic saline at 1 ml./min had no effect on feeding behaviour.

RESULTS

Portal vs. jugular vein infusion of a mixture of short-chain fatty acids

A mixture of 0·55 M-sodium acetate: 0·30 M-sodium propionate: 0·15 M-sodium butyrate (B.D.H., Poole, Dorset), which is typical of the molar proportions in rumen fluid, was infused at rates of 1, 2 or 4 m-mole/min; six sheep were used and the treatments were given in random order. The weight of food eaten during the infusion of 4 m-mole/min infusion of the mixture into the portal vein was 32 ± 26 g ($n = 5$), whereas with jugular infusion at the same rate the intake was 80 ± 13 g ($n = 5$). Both of these were significantly lower ($P < 0{\cdot}001$) than the 189 ± 12 g ($n = 44$) which was eaten on intervening control days, and infusion into the portal vein depressed intake significantly more than into the jugular vein ($P < 0{\cdot}05$).

With an infusion rate of 2 m-mole/min intakes during portal and jugular vein infusions were 111 ± 43 g ($n = 5$) and 167 ± 37 g ($n = 6$), while during 1 m-mole/min infusion into the portal vein 147 ± 39 g were eaten.

Cumulative intakes at 30 min intervals for the portal vein infusions are shown in Fig. 1. Some food was usually eaten during the first 30 min of infusion, even with 4 m-mole/min intraportally, where feeding was almost completely suppressed during the remainder of the infusion period. Feeding recommenced during the hour after the infusion was stopped and by the next morning the lower food intake during infusion had been completely compensated for, there being no effect on the 24 hr food intake. There was no effect of any treatment on rectal temperature.

Portal vein infusions of individual short-chain fatty acids

In an endeavour to see whether any one of the three short-chain fatty acids used in the mixture in Experiment 1 was particularly responsible for the intake-depressing effects, separate infusions were made into the portal or jugular vein of 2·21 m-mole/

min of sodium acetate, 1·20 or 0·60 m-mole/min of sodium propionate or 0·60 m-mole/min of sodium butyrate. Nine sheep were used, three of which had been used in Experiment 1.

The mean weight eaten during the 3 hr infusion period on control days was 205 ± 33 g. Intakes during portal infusions of acetate, propionate and butyrate at their rates of inclusion in the 4 m-mole/min of mixture in Experiment 1 were

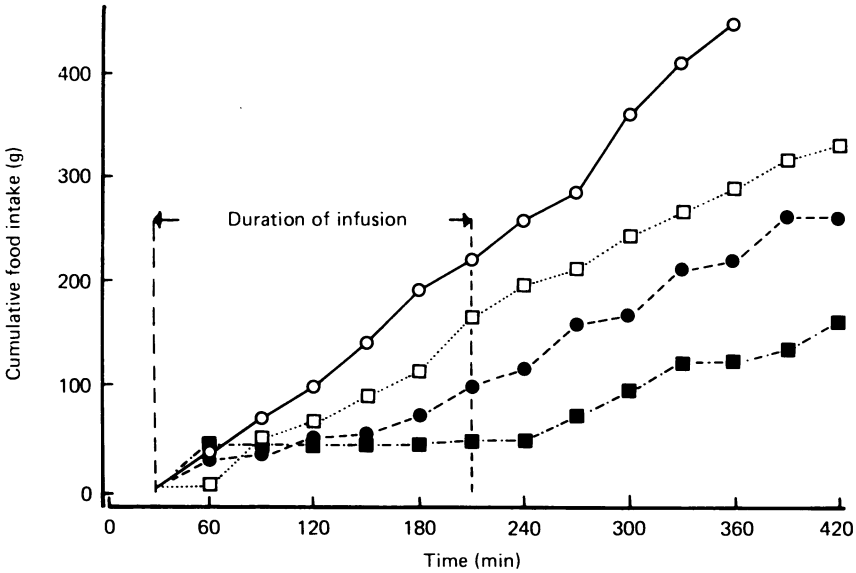

Fig. 1. Cumulative food intake of sheep infused for 3 hr into the hepatic portal vein with mixtures of short-chain fatty acids. ○, saline control; □, 1 m-mole/min; ●, 2 m-mole/min; ■, 4 m-mole/min.

152 ± 35 g (n = 5), 39 ± 91 g (n = 11) and 471 ± 288 g (n = 3), respectively, the propionate effect being significant (P < 0·001). Jugular vein infusions at the same rates did not affect feeding (188 ± 39 g, n = 3, eaten during 2·21 m-mole/min of acetate; 210 ± 89 g, n = 7, during 1·2 m-mole/min of propionate). Propionate infusion at its rate of inclusion in the 2 m-mole of mixture/min in Experiment 1 (0·6 m-mole/min) slightly depressed intake when given intraportally (150 ± 58 g, n = 6, n.s.) and had no effect when given into the jugular vein (220 ± 51 g, n = 6). Acetate (2·21 m-mole/min) intrajugularly had no effect on the weight eaten during infusion (188 ± 39 g, n = 3).

An example of the feeding behaviour of two sheep on control and treatment days is shown in Fig. 2. It will be seen that no food was eaten during the propionate infusion in this case.

Denervation of the liver and the feeding response to portal vein infusions of propionate

It was shown in Experiment 2 that the effect of portal infusions of a short-chain fatty acid mixture on feeding was due to its propionate content. In order to investigate the role of the liver in this response, partial denervation of the liver was followed by infusion of sodium propionate at 2·1 m-mole/min into the portal vein (Fig. 3).

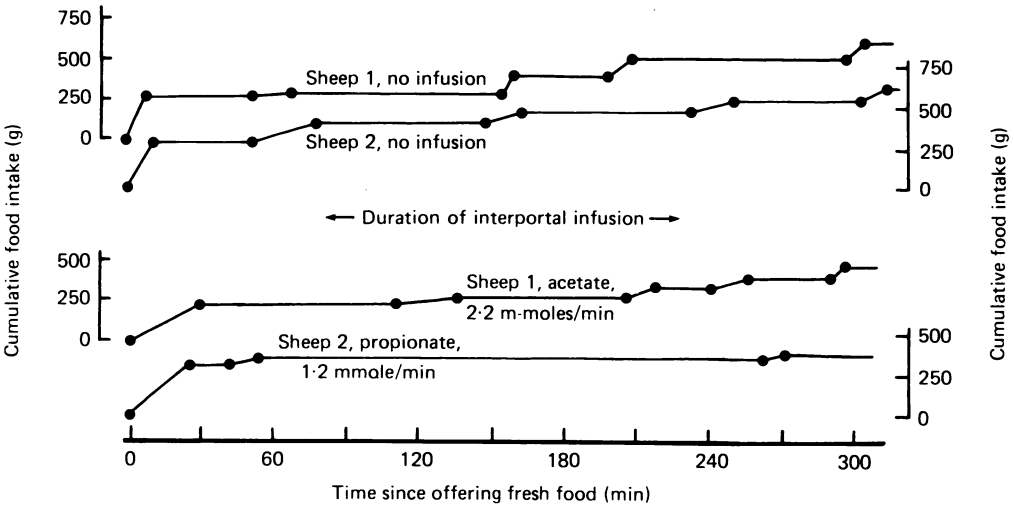

Fig. 2. Cumulative food intakes of two sheep either without infusion or with intraportal infusion of sodium acetate or sodium propionate.

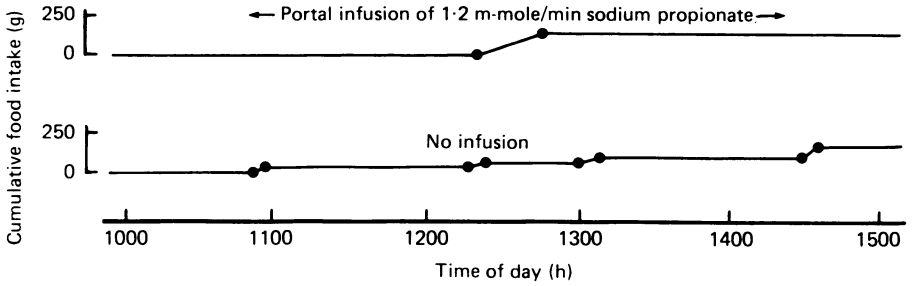

Fig. 3. Cumulative food intakes of a sheep with section of the hepatic plexus (no. 17) during infusion of sodium propionate into the portal vein (upper trace) and on the next day, with no infusion (lower trace).

Surgery was performed on ten sheep, seven of which recovered pre-operative levels of food intake. Propionate infusions were made for 3 hr, usually starting 30–60 min after offering fresh food. Two animals did not eat significant quantities of food during the equivalent period on control days, however, and some 3 hr infusions were performed later in the day.

Table 1 summarizes the results obtained from the seven sheep. Three animals (nos. 1, 2, 9) did not eat during portal infusion of propionate except in the first few minutes: that is, they responded in a similar manner to intact animals. On histological examination of the operated area these three sheep were found to have been incompletely denervated (e.g. Pl. 1*B*). Three sheep (nos. 3, 17, 26) continued to eat meals during infusion in a similar manner to control days; denervation was found to be complete or almost complete in these animals (e.g. Pl. 1*C*). One animal (no. 12) took small meals during some infusions but ate nothing during

other infusions; histology showed the hepatic plexus to be approximately half sectioned in this sheep.

The total daily food intakes of all sheep were within the normal range (1·0–1·5 kg/day). Two denervated animals (nos. 12, 17) ate three or four very large meals daily whereas the other sheep ate more typically, that is ten to twelve meals per day.

TABLE 1. Summary of results from sheep which recovered normal food intake after attempted section of the hepatic plexus

Animal no.	Feeding response to intraportal propionate	Extent of denervation assessed histologically
1 (♂)	Feeding inhibited (5 infusions)	Partial (75%) (Pl. 1*B*)
26 (♂)	Ate normally (3 infusions)	Complete (95%) (Pl. 1*C*)
12 (♂)	Ate small meals (5 infusions) Feeding inhibited (4 infusions)	Partial (50%)
3 (♀)	Ate normally (5 infusions)	Complete (95%)
2 (♂)	Feeding inhibited (4 infusions)	Partial (25%)
9 (♀)	Feeding inhibited (4 infusions)	Partial (33%)
17 (♀)	Ate normally (5 infusions)	Complete (100%)

DISCUSSION

Intravascular infusions of volatile fatty acids have been shown to depress food intake (Dowden & Jacobson, 1960; see review by Baile & Forbes, 1974) both for continuous infusion into the jugular vein and for infusions into several blood vessels during spontaneous meals. Neither of these protocols is physiological in that absorption of the volatile fatty acids produced by fermentation of a meal takes place over a period of hours following the meal rather than a high rate during the period of feeding only, and the route of entry is via the portal vein rather than into the systemic circulation. In particular, the liver removes a large proportion of the propionate and prevents it entering the general circulation. The procedure of continuous infusion of physiological quantities of the salts of volatile fatty acids into the portal vein, as adopted in the experiments reported in this paper, should yield more useful results than those previously reported. Sodium propionate, but not acetate or butyrate, effectively prevented feeding from 30 min after the start of infusion until after the end of infusion when given into the portal vein at 1·2 m-mole/min. The fact that increases in the quantity of propionate flowing in the portal vein of the same order occur following feeding (Thompson, Bassett & Bell, 1978) suggests that the response to propionate, located in or near to the liver, may be involved in the natural control of feeding behaviour.

A question then arises as to whether the signal from the liver to the central nervous system is nervous or blood-borne. The three sheep in which denervation of the hepatic plexus was at least 95% complete ate normally during infusions of sodium propionate at a rate which completely inhibited feeding in intact sheep. Three of the sheep in which 75% or less of the hepatic plexus was sectioned did not eat during similar infusions; one animal which was approximately 50% denervated gave equivocal results. These results show that the effect of propionate on feeding

behaviour, previously postulated to be mediated by the liver, is blocked by section of most of the hepatic afferent fibres. This is comparable with the results of Novin and his colleagues (see Novin & Vanderweele, 1977), who found that subdiaphragmatic section of the vagus prevented the intake-depressing effects of portal glucose infusions in rabbits. Much of the portal content of propionate is converted to glucose in the ruminant liver (Bergman, 1975) suggesting that the mechanism of the propionate effect in the sheep might be similar to that of the effect of glucose in the rabbit.

The fact that feeding during portal propionate infusion was approximately normal in the three liver denervated sheep suggests that there are no other major sites in the body which mediate the effect of propionate on feeding. This is not to imply that there are no receptors in other organs for other metabolites (e.g. acetate or pH receptors in the rumen wall), or for gut distension. Such other receptors must indeed be postulated if we are to explain the the normal size and distribution of meals in two of the denervated sheep which ate during infusions (nos. 3, 26) and the normal daily food intakes in all four animals whose response to propionate was partly or completely blocked.

It is likely that there are receptors in various organs, including the brain itself, sensitive to the many factors which change as a result of eating food. The dominant signal at any given time will depend on circumstances, particularly the rate at which potentially inhibitory metabolites can be removed from the circulation by non-receptor tissues and the rate at which gut-distending bulk can be digested or voided.

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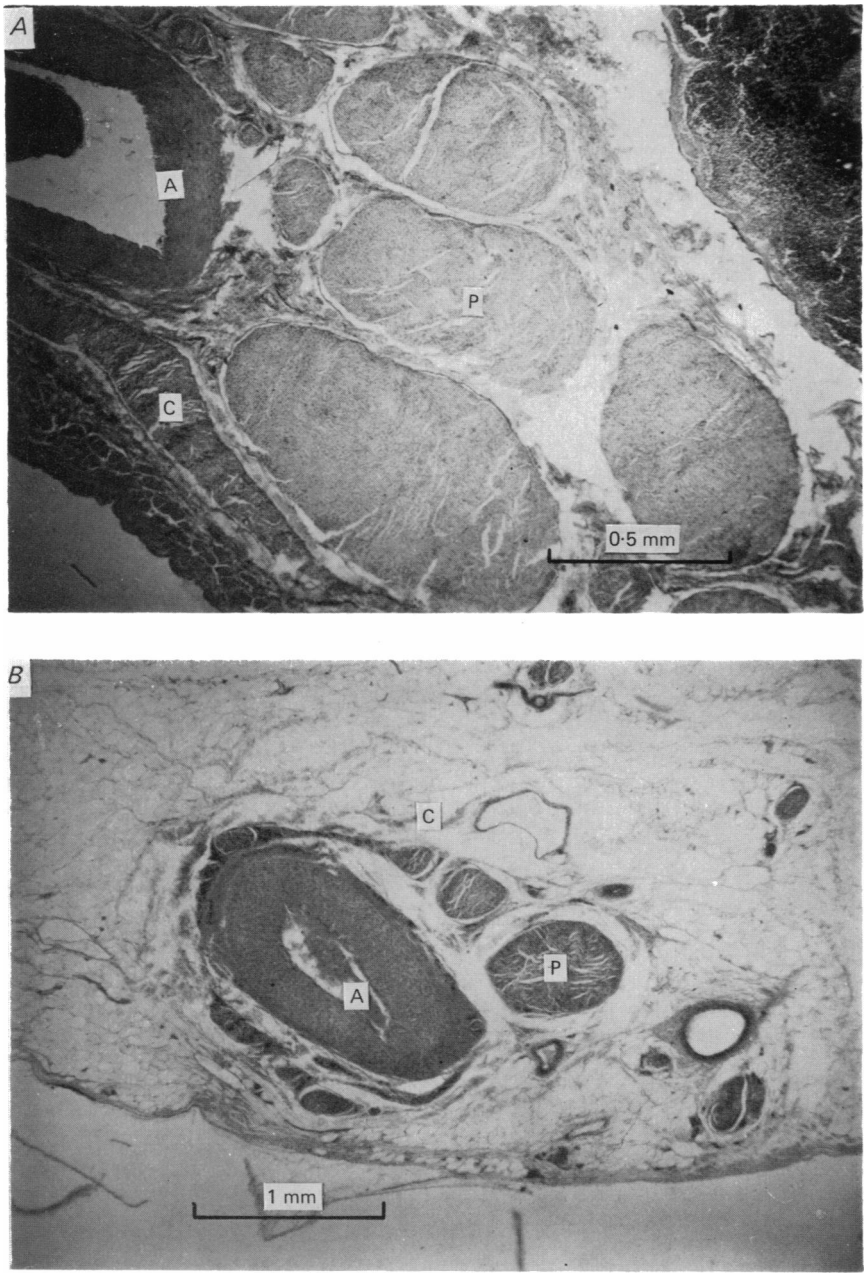
EXPLANATION OF PLATE

PLATE 1

A, cross-section of hepatic artery and adjacent nerve bundles in an intact sheep.
B, hepatic artery showing partial denervation (no. 1).
C, hepatic artery showing complete denervation (no. 26).
A, hepatic artery; *C*, connective tissue; *P*, hepatic plexus.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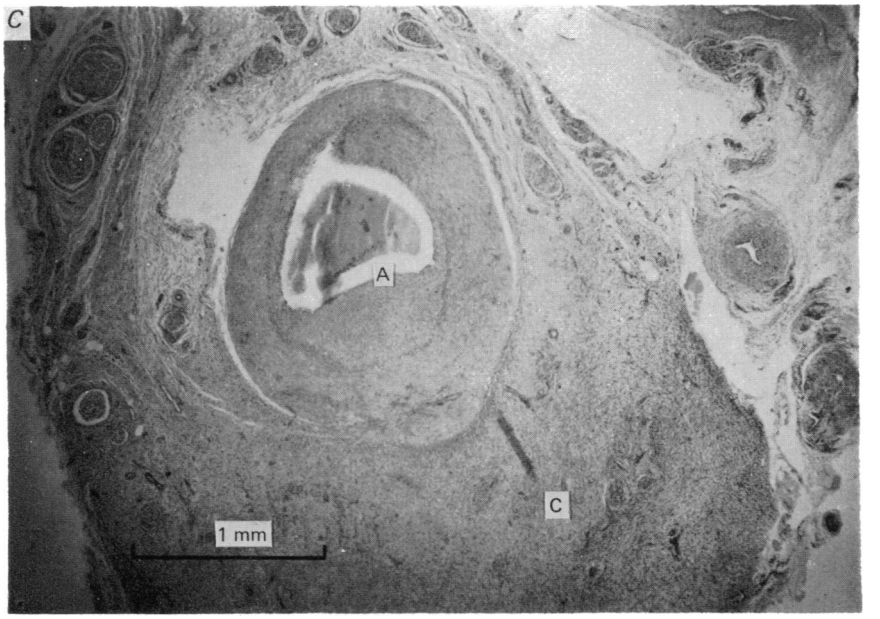
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