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STUDIES  
ON THE ABSORPTION OF VOLATILE FATTY ACIDS  
IN CALVES

A thesis presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Nutrition at Massey University of Manawatu.

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## INTRODUCTION

Ruminants subsist on fibrous plant feeds not normally consumed by man. They transform inedible and undigestible plant material into palatable and digestible human food. Their meat and milk are rich sources of essential amino-acids, vitamins and minerals. These foods are natural supplements to the human diet which is predominantly of plant origin.

Interest in the nutrition and digestive physiology of ruminants has resulted in the accumulation of a voluminous literature which has been reviewed by Annison and Lewis (1959), Barnett and Reid (1961), Blaxter (1962) and Kay and Hobson (1963). This interest has also prompted two recent international symposia, the proceedings of which have been edited by Lewis (1961), and Dougherty, Allen, Burroughs, Jacobson and McGilliard (1965).

Mature ruminants are assumed to have the same basic nutritional requirements for energy, amino-acids, vitamins, minerals and water as do the simple-stomached animals. Their digestive juices, like those of all other mammals, do not contain any cellulases. An extensive microbial population, located largely within the rumen, and to a lesser extent in the caecum and colon, enables them to thrive on fibrous plant feeds that are frequently deficient in some of the essential amino-acids and water-soluble vitamins.

Ingested solid feeds enter directly into the rumen, where they are held and fermented. Initially cellulolytic micro-organisms erode the cell walls of plant material and expose the cellular contents to continued fermentation. Only a small proportion of dietary nutrients survive the

microbial activity of the rumen. Proteins are first hydrolyzed into amino acids, short-chain fatty acids and ammonia. These degradation products, together with the non-proteinaceous nitrogenous compounds of the feed and saliva, are then resynthesized into bacterial and protozoal proteins. The amino-acid requirements of ruminants are believed to be met by the digestion and absorption of these microbial proteins in the distal portions of the alimentary tract in a manner similar to that found in simple-stomached animals. Among the products of rumen fermentation are the steam volatile fatty acids (referred to from here on as the VFAs), water-soluble vitamins and vitamin K. The VFAs are absorbed directly from the rumen and serve as the major source of energy in ruminants (Carroll and Hungate, 1954; Balch, 1958; Blaxter, 1962). Essential amino-acids, water-soluble vitamins and vitamin K are apparently produced in sufficient quantities in the rumen to meet nutritional requirements, since under normal feeding conditions mature ruminants are not known to suffer from any of the deficiency diseases associated with these nutrients in simple-stomached animals.

#### The Rumen in Adult Bovines

The fully-developed rumen occupies most of the left half of the abdominal cavity and its ventral aspect extends well beyond the median plane (Sisson and Grossman, 1953). In mature cattle the ruminoreticular volume, as determined by its capacity to hold water, ranges from 100-200 lit and represents approximately 85 % of the total stomach capacity (Warner and Flatt, 1965). The rumen is lined with a non-glandular, keratinized, squamous epithelium which is thrown up into numerous folds and papillae. Although this epithelium is incapable of secreting any digestive enzymes, its vascularization and histological structure indicate an absorptive capacity (Dobson, Brown, Dobson and Phillipson, 1956).

Three major blood vessels drain the rumen, namely, the right ruminal, the left ruminal and reticular veins. These veins are valved and receive contributions from other parts of the viscera. All three veins eventually combine to form the gastric vein, which is deeply embedded in the connective tissue of the portal fissure. This vessel in turn joins the anterior mesenteric vein, which collects the effluent blood from the entire intestines with the exception of part of the duodenum and rectum, to form the portal vein (Grossman and Sisson, 1953).

The rumen has a constant temperature of 39°C and offers a near-ideal environment for the continuous growth and development of anaerobic microbial populations (Annison and Lewis, 1959). Nutrients, water and saliva enter the rumen and metabolic end-products are prevented from accumulating by eructation, passage of fermented food to the distal portions of the alimentary tract, and absorption through the rumen wall. This seemingly simple symbiotic relationship between host and microbial populations is coordinated by a number of complex physiological phenomena that include the regulation and control of feed and water intake, absorption and utilization of fermentation products, eructation of gases, rumination and rumen movements, secretion of saliva and pH changes of rumen contents.

The VFAs (acetic, propionic and butyric acids) are among the most important products of rumen fermentation. Although both soluble and insoluble carbohydrates are the major sources of these acids, they can also be produced from the protein and lipid fractions of the diet as well (Annison and Lewis, 1959; Barnett and Reid, 1961). McAnally and Phillipson (1942) first demonstrated the transfer of these acids across the rumen wall by their appearance in rumen effluent blood of anaesthetized sheep. Annison and Lewis (1959), Barnett and Reid (1961), Dobson (1961),

Blaxter (1962), Warner (1964) and Annison (1965) have reviewed the literature concerning their production and absorption.

#### The Rumen in Calves at Birth

The rumen in calves at birth is small and undeveloped, and is tucked away in the anterior dorsal aspect of the abdominal cavity (Tamate, McGilliard, Jacobson and Getty, 1962; Sisson and Grossman, 1953). Rumen volume ranges from 0.5-1.6 lit, and represents approximately one third of the total stomach capacity (Warner, Flatt and Loosli, 1956; Tamate et al., 1962). The rumen wall is thin and the papillae on its inner surface are short and undeveloped (Warner et al., 1956; Tamate et al., 1962). Brownlee (1956) and Warner et al. (1956) observed a high degree of papillary development in calves fed on high-concentrate low-fiber diets and suggested that the products of rumen fermentation act as the primary stimuli for rumen development. This hypothesis has since been confirmed and the active principles identified as the VFAs (Flatt, Warner and Loosli, 1958; Sander, Warner, Harrison and Loosli, 1959; Tamate et al., 1962). The experiments of Harrison, Warner, Sander and Loosli (1960) further indicated a regression of rumen papillae in calves that were changed from a diet containing hay and concentrates to one that was composed of milk only, thus indicating that an active rumen fermentation was essential for the maintenance of rumen papillae. Warner and Flatt (1965) have reviewed the literature dealing with the anatomical development of the ruminant stomach in a paper presented at the Second International Symposium on the Physiology of Digestion in the Ruminant.

Calves are almost entirely dependent on colostrum and milk for their

supply of nutrients during the first few days of post-natal life. These feeds enter directly into the abomasum (Watson, 1944; Comline and Titchen, 1951, 1961), and are digested and assimilated in a manner similar to that found in simple-stomached animals (Tagwerker, 1961). Numerous studies have been conducted on the nutrition of the calf and on the functional development of the rumen. The results of these studies were reviewed by Tagwerker (1961), Preston (1963), Kay and Hobson (1963) and Roy (1964). There is a paucity of information however on the absorption of VFAs from the rumen of young calves.

Volatile fatty acid absorption in young ruminants has been studied in conscious and anaesthetized preparations either by the disappearance of these acids from the rumen or by their appearance in the blood. The advantages and limitations of both methods have been discussed by Annison and Lewis (1959). Warner (1964) and Annison (1965) have recently reviewed the literature on the ruminal production and absorption of VFAs. It is apparent from these reviews that there are no reliable methods as yet for the quantitative estimation of VFAs produced in and absorbed from the rumen.

#### Volatile Fatty Acid Absorption Studies in Calves Based on the Analysis of Rumen Contents

Mature VFA concentrations were demonstrated in the rumen of 3-4 week-old pasture-reared calves by Godfrey (1961) and Stewart (1962). Neither of these workers however attempted to determine whether the VFAs were absorbed at this early age or not.

Flatt, Warner and Loosli (1956) investigated the absorption of VFAs at different ages in ten rumen-fistulated Friesian calves reared on a diet

of milk, hay and calf starter. They introduced warm equimolar solutions (10 g/lit) of butyric, propionic and acetic acids, buffered to a pH of 6.2, into the emptied rumen and measured the losses that occurred over a period of 60 minutes. These experiments were performed when the calves were 3, 5, 7, 11 and 15 weeks old. The authors averaged the results of all fifty trials and concluded that 30.2 m-moles of butyric acid, 30.6 m-moles of propionic acid and 28.2 m-moles of acetic acid were lost from the rumen per hour. They did not however include in the publication the absorption rates determined at the different ages, neither did they mention the method by which VFA losses were estimated.

#### Volatile Fatty Acid Absorption Studies in Calves Based on the Analysis of Blood

In a series of three separate experiments, McCarthy and Kesler (1956) observed an increase in the VFA concentration of jugular blood with advancing age in calves. Six Friesian bull-calves were used in each of the three trials. The calves were allowed at least 2 days of colostrum feeding on their dams, and by the age of 5 days were on experiment. They had free access to both hay and calf starter, besides receiving 5 lb of either whole herd milk (Trials I and II) or milk replacer (Trial III). All the calves were weaned at 6 weeks of age. Jugular blood samples were collected at weekly intervals approximately 3 hours after the morning feeding for VFA determination. The mean VFA concentration of jugular blood of all eighteen calves increased gradually from a level of 0.30 m-moles/lit at the age of 1 week to 1.1 m-moles/lit at 15 weeks of age. McCarthy and Kesler associated this increase with the development of rumen function.

Martin, Ramsey, Matrone and Wise (1959) investigated the ability of young Friesian bull-calves to utilize the preformed salts of the VFAs. They compared the growth rates and post-prandial VFA concentration changes of jugular blood at the age of 3, 6, 9 and 12 weeks in three groups of calves fed on different iso-caloric diets. The diet of Group I calves was composed of casein, fat, glucose, starch and chopped hay; that of Group II contained casein, fat, glucose and the salts of acetic, propionic and butyric acid; the calves in Group III were reared on whole milk only. Nine Friesian bull-calves were used in this experiment. The calves were randomly assigned to one of the three experimental groups after 2-3 days of colostrum feeding. Whole milk was fed to all the calves at the rate of 10 % of body weight per day. The solid diets were introduced to Group I and Group II calves at the age of 5 days, and milk was gradually reduced during the third week. These calves were weaned onto the experimental diets by the end of the third week. Group III calves were continued on the same rate of milk feeding. All feeds were offered twice daily. Jugular blood samples were collected at weekly intervals before and 3 and 6 hours after feeding. Marked post-prandial elevations in the levels of jugular blood VFAs were noted in Group I and Group II calves, but not in those fed on milk only. The magnitude of these elevations increased with advancing age, and was invariably greater in the calves fed on the diet containing the salts of the VFAs (Group II) than in those receiving no preformed VFAs (Group I). Since the mean daily weight gains were 0.37, 0.45 and 0.62 kg/calf/day for Group I, Group II and Group III calves respectively, the authors concluded that the rumen was capable of VFA absorption at this early age. They further suggested that the increasing post-prandial concentrations of jugular VFAs observed with advancing age in Group I and Group II calves reflected an improving efficiency of VFA absorption, and

attributed it to the functional development of the rumen.

The pre-feeding concentrations of jugular blood VFAs in the experiment of Martin et al. (1959) were approximately 0.3 m-moles/lit, and were unaffected by either the age of the calves or their diet. An increase in the level of jugular blood VFAs from 0.3 m-moles/lit at 1 week to approximately 1.1 m-moles/lit at 15 weeks of age was however noted in the experiments of McCarthy and Kesler (1956). This apparent discrepancy may have been due to the fact that the calves in the former experiment were fed twice daily and were sampled before feeding, while those in the latter experiment had had free access to solid feeds and were sampled 3 hours after feeding. Martin and his co-workers were thus investigating pre-feeding levels, while McCarthy and Kesler were determining post-prandial concentrations. Dinda, cited by Preston (1963) could not establish a relationship between the VFA concentration of jugular blood and the age of early-weaned calves. He reported that the VFA levels in jugular blood ranged from 1-2 m-moles/lit.

Conrad, Smith, Vandersall, Powden and Hibbs (1958) determined the gastrosplenic blood flow rate in seven 4-7 month-old anaesthetized calves by the isotope dilution technique. They then collected post-prandial serial samples of both gastrosplenic and jugular blood samples from three other 4-5 month-old conscious calves fed on a 2:1 mixture of alfalfa hay and a grain concentrate. By subtracting the jugular VFA concentrations from the corresponding gastrosplenic levels and using the mean gastrosplenic blood flow rate determined in the initial acute experiments (763.7 ml/min/100 lb body weight) these workers estimated that 29 % of the total calories digested were absorbed in the form of acetate and propionate.

No attempt was made in any of these studies to investigate the absorption of VFAs from the rumen during the first two weeks of age prior to the consumption of solid feeds. All the evidence suggests that the transport of VFAs across the rumen wall is dependent on the consumption of solid feeds and on the establishment within the rumen of an active microbial fermentation. Preston (1963) in his review on the nutrition of the early-weaned calf concluded:-

"it is axiomatic that if 75 per cent of the dry matter of concentrates or grass is digested by the 4-week-old calf, and that the mode of digestion is by microbial fermentation, then the main products of such breakdown, namely the steam-volatile fatty acids, must be absorbed and utilized as sources of energy even by the very young calf."

In reviewing the recent advances in ruminant physiology, Kay (Kay and Hobson, 1963) stated:-

"Both the ability to absorb fatty acids from the rumen and salivary secretion only begin to develop in the very young ruminant with the onset of rapid fermentation in the rumen."

On the basis of these two conclusions the apparent dependence of VFA absorption from the rumen on the consumption of solid feeds during early life suggests the post-natal establishment of new mechanisms for the transport of these acids across the rumen wall. A better understanding of the VFA absorptive capacity of the rumen during this crucial stage of development is not only of academic interest, but is also essential for the development of better and more economical calf feeding and management practices. The experiments described in this Thesis were therefore aimed at:-

1. Determining whether calves are capable of absorbing VFAs from the rumen during the first few days of life prior to the consumption of solid feeds.
2. Determining the effects of advancing age and diet on the absorption of VFAs from the rumen in young calves.

One year after these studies were initiated, Sutton, McGilliard and Jacobson (1963) presented evidence to indicate that "the ability to absorb large quantities of acetic acid is not inherent in the rumen and does not develop in calves on a diet of milk only." They used two pairs of conscious Friesian calves to investigate the effects of advancing age and diet on the absorption of acetic acid from the rumen. One member of each pair was reared on a diet of milk only and the other was fed hay and calf starter in addition to milk. Acetic acid absorption was estimated by changes in the concentration of this acid relative to polyethylene glycol (a marker) following the introduction into the rumen of a buffered solution containing known concentrations of these two compounds. Using this experimental approach, they demonstrated "an absence of effective absorption in very young calves and in older milk fed calves" and suggested that "the large increase in the rate of absorption which was demonstrated to accompany maturation of rumen structure must be due, not to the development of new mechanisms but to the expansion of existing ones."

Although Sutton and his co-workers estimated the disappearance of acetate from the rumen, they were unable to detect its appearance in the peripheral blood of the first pair of calves, and could not interpret their data on jugular blood analysis. They did not include these results in their publication and discontinued the sampling of jugular blood from

the second pair of calves.

Volatile fatty acid absorption from the rumen was investigated in the experiments described in this Thesis by changes in the concentrations of these acids in the blood. A logical approach to establish base lines lay in comparing the fasting and post-prandial concentrations of blood VFAs at regular intervals with advancing age in two groups of calves, one reared on a diet of milk only, and the other having, besides milk, free access to pasture. Rumen effluent blood was considered to be the most satisfactory for the purposes of this experiment. The small size and inaccessibility of ruminal veins in young calves, however, precluded the sampling of this blood in conscious preparations. Although portal blood is prone to laminar flow (Annison, Hill and Lewis, 1957), and at best represents diluted rumen effluent blood, recourse to the portal vein seemed to be the most practical approach, especially since Schambye (1951 a & c) and Annison et al. (1957), demonstrated marked hepatic removal of absorbed VFAs from the peripheral circulation.

S E C T I O N I

VOLATILE FATTY ACID ABSORPTION STUDIES

IN

CONSCIOUS CALVES

## CHAPTER I

### POST-PRANDIAL CHANGES IN THE VOLATILE FATTY ACID AND GLUCOSE CONCENTRATIONS OF PORTAL BLOOD IN CONSCIOUS CALVES

The effects of advancing age and diet on the absorption of VFAs from the rumen were investigated, in the experiments described in this chapter, by the analysis of portal blood and rumen liquor. Plans originally called for:

1. The establishment of a portal vein catheter and a rumen fistula in 5-10 day-old calves.
2. The rearing of one group of these calves on a diet of milk only, and another group on a diet of milk and pasture.
3. The determination of fasting and post-prandial concentrations of circulating glucose and VFAs in the portal blood of these calves as soon after surgery as was practicable, and at weekly intervals with advancing age thereafter.

Blockage of the portal vein catheters limited the application of this experimental approach to a period of two weeks after the surgical introduction of the catheters. Experiments were therefore conducted in calves of various ages and in different stages of rumen development within a few days after the surgical insertion of a catheter into the portal vein.

REVIEW OF LITERATURE

Volatile Fatty Acid Absorption Studies in Mature Conscious Ruminants Based on the Analysis of Portal Blood

Schambye and Phillipson (1949) were the first to investigate VFA absorption from the alimentary tract to the blood in conscious ruminants. These workers used mature sheep which had previously been provided with a rumen fistula, an exteriorized carotid artery and a London cannula adjacent to the main trunk of the portal vein. They collected serial samples of rumen liquor, carotid arterial blood and portal venous blood at regular intervals after the feeding of a test diet composed of chopped hay, linseed meal and blood meal, and demonstrated marked post-prandial elevations in the VFA concentrations in both rumen liquor and portal blood. Portal VFAs were observed to be consistently higher than the corresponding carotid levels, and this veno-arterial concentration difference became more pronounced as the concentration of the VFAs within the rumen increased. Although similar post-prandial changes in the levels of circulating glucose were noted, the relative magnitude of these changes, when expressed on a molar basis, was considerably smaller than that observed with the VFAs. The authors concluded that these observations indicated both VFA absorption from the alimentary tract and VFA metabolism in the body.

The above-mentioned preliminary observations were confirmed in a series of experiments with mature conscious sheep in which a London cannula was again used to sample the portal vein (Schambye, 1951 a & b). Schambye (1951 c) also showed that little, if any, glucose is absorbed from the alimentary tract of mature sheep. His acute experiments in sheep (Schambye, 1951 a) indicated hepatic removal of a large portion of absorbed dietary

acetate from the portal circulation. He later used Evans Blue and P<sup>32</sup> as markers to determine the rate of blood flow through the portal vein of sheep (Schambye, 1955 a & b), and combined the results of these experiments with the post-prandial VFA concentration changes of the portal blood determined in his earlier experiments (Schambye, 1951 a & b) to calculate the amounts of VFAs absorbed daily.

Annison, Hill and Lewis (1957) used the surgical technique developed by Lewis, Hill and Annison (1957) to establish portal vein catheters in sheep, and determined the post-prandial glucose and VFA concentration changes in the portal and peripheral blood following the feeding of various test diets. These workers confirmed Schambye's earlier observations (Schambye, 1951 a, b & c; 1955 a & b) and by chromatographic separation of the VFA fraction of both portal and peripheral blood demonstrated near complete hepatic removal of absorbed propionate from the portal circulation. They also calculated the quantity of propionic acid metabolized by the liver on various diets over a 24-hour period by using the portal blood flow rate of 37 ml/min/kg of body weight determined by Schambye (1955 a), and by assuming that the portal blood propionate concentration at 8-10 hours after feeding was representative of the whole period.

Blood flow through the portal vein of ruminants was investigated by Schambye (1955 a), Fegler and Hill (1958), Fries and Conner (1961), and Bensadoun and Reid (1962). These workers demonstrated a reduction in portal vein blood flow following fasting, anaesthesia and surgery. Their experiments suggest that VFA absorption can be grossly underestimated if investigated in acute preparations, or if portal blood flow rates that were determined in anaesthetized preparations are used in conscious animals.

Bensadoun and Reid (1962), working with sheep, and Waldern, Johnson and Blosser (1963) with cattle, demonstrated post-prandial increases in portal vein blood flow which were observed to reach a peak approximately 6 hours after feeding. Their findings indicate that misleading results can be obtained in absorption studies if a single or an average portal vein flow rate value is used to represent the whole period under study.

### Volatile Fatty Acid Absorption Studies in Young Conscious Calves Based on the Analysis of Portal Blood

No reference was found in the literature consulted to any published work based on the analysis of portal blood concerning the absorption of VFAs from the rumen of young conscious calves. The experiments of Conrad et al. (1958) and Waldern et al. (1963) were performed on calves more than four months old.

## MATERIALS AND METHODS

### Experimental Design

Post-prandial changes in the glucose and VFA concentrations of portal blood were investigated in four Friesian bull-calves that had had at least 2 days of colostrum feeding on their dams. Calves 1 and 2 were reared on milk only and were housed in concrete calf pens where slatted wooden frames constituted the only bedding (Milk Fed Calves). Calves 3 and 4 were raised on pasture - a mixture of white clover, short-rotation and long-rotation rye grass - from the age of 6 days onwards (Pasture Fed Calves). Warm whole milk was bucket-fed twice daily to all the calves at the rate of 4.5 lit/calf/day.

A rumen fistula was prepared in calves 2, 3 and 4, when they were 6, 55 and 40 days old respectively, and a cannula was inserted into the portal vein a few days before the calves were to be placed on experiment. Calves 3 and 4 were weaned onto pasture at the age of 62 and 70 days respectively. The sequence of these events is summarized in table 1.

TABLE 1

Rearing and surgical details of calves used in milk and pasture feeding absorption experiments.

Calf	Diet	Age at weaning days	Weight at weaning kg	Age rumen fistulated days	Age portal catheterized days
1	Milk	-	-	-	6
2	Milk	-	-	6	33
3	Milk & Pasture	62	77	55	75
4	Milk & Pasture	70	75	40	111

Each experiment was preceded by a period of fasting which was followed by the feeding of either milk or pasture. Serial samples of portal blood were collected before and at regular intervals after feeding. Simultaneous samples of rumen liquor and jugular blood were also collected, whenever possible, in calves 2, 3 and 4. Experiments following milk consumption were performed on all four calves, and after grazing on calves 3 and 4 only. Details of the age of the calves, the duration of the preliminary fasting periods, the quantity of milk consumed and the time spent grazing are presented in table 2.

TABLE 2

The age, weight, duration of fasting, quantity of milk consumed and time spent on pasture in milk and pasture feeding absorption experiments.

Calf	Treatment	Milk feeding experiments				Pasture feeding experiments			
		Age days	Weight kg	Fasting hr	Milk consumed lit	Age days	Weight kg	Fasting hr	Time on pasture hr
1	Milk	9	41	23	2	-	-	-	-
2	Milk	36	48	24	4	-	-	-	-
3	Milk & Pasture	83	83	24	4.5	78	83	24	3½
4	Milk & Pasture	114	94	48	4.5	115	94	72*	3¼

\*Restricted from pasture for 72 hr but given 4.5 lit of milk 48 hr after the beginning of starvation.

## Surgical Methods

The serial sampling of portal vein blood, jugular vein blood and rumen liquor from conscious calves was made possible by previous catheterization of the portal and jugular veins, and fistulation of the rumen. Catheters were introduced into the jugular vein under local skin anaesthesia. Rumen cannulae and portal vein catheters were inserted under general anaesthesia.

Anaesthesia. Surgery was preceded by a 24-hour period of fasting off milk and 48 hours off pasture. Pre-anaesthetic medication consisted of the intravenous injection of 50-75 mg of chlorpromazine and chlorpromazine hydrochloride (Largactil, May and Baker). Anaesthesia was induced 10 minutes later by the intravenous injection of 150-240 mg of pentobarbitone sodium (Nembutal, Abbott Laboratories), and was maintained with chloroform (May and Baker), administered by an open mask technique.

Surgical procedure for the fistulation of the rumen. The abdominal cavity was entered on the left side through a 7-10 cm paracostal skin incision starting approximately 2 cm posterior to the last rib and 3 cm ventral to the dorsal border of the left paralumbar fossa. A Jarrett cannula (Jarrett, 1948) was introduced through this incision into the most caudad region of the dorsal sac of the rumen and was held in position with a linen purse-string suture. Eight equidistant, circumferentially disposed suspension sutures were passed through the rumen wall at a distance of 0.5 cm from the neck of the cannula, and were exteriorized ahead of the cannula through a separate circular skin excision located in the dorsal aspect of the left paralumbar fossa approximately 3 cm posterior to the original skin incision. Before sewing the suspension

sutures to the skin 6-8 interrupted sutures were used to sew peritoneum to muscle layers around the border of the circular excision. The original abdominal incision was then closed. A plastic washer was placed around the neck of the cannula to prevent it from retracting into the rumen.

Surgical procedure for the catheterization of the portal vein. The surgical procedure developed by Conner and Fries (1960) was used to cannulate the portal vein. Oral administration of warfarin sodium (Marevan, Evans Medical) - a prothrombenopenic drug having a latent period of activity of 28-36 hours - was started 18-20 hours preoperatively in order to retard the development of post-operative thrombophlebitis. Initial loading doses of 10 mg for Milk Fed Calves and 30 mg for Pasture Fed Calves were given.

Post-operative medication. Prophylactic treatment with antibiotics was started upon the completion of surgery and was continued for 4-6 days post-operatively. This entailed the daily intramuscular injection of one million units of penicillin (Benethamine Penicillin, Glaxo Laboratories), and the dusting of surgical wounds with neomycin and bacitracin powder (Cicatrin, Calmic).

In the case of portal vein catheters a fibrinolytic preparation (Varizyme, American Cyanamid) was intravenously administered at the rate of 12,500 units per day for a period of 3-5 days post-operatively. Oral medication with warfarin sodium (Marevan, Evans Medical) was continued at a daily dosage of 2.5 mg for Milk Fed Calves and 10 mg for Pasture Fed Calves.

Care of portal vein catheters. The following precautions were taken

to retard the development of post-operative thrombophlebitis:-

1. Post-operative dosing with prothrombenopenic (Marevan, Evans Medical) and fibrinolytic (Varizyme, American Cyanamid) preparations.
2. Twice-daily withdrawal of blood when the catheters were not in use.
3. Routine flushing of the catheters with 0.9 % NaCl following the withdrawal of blood.
4. Keeping the catheters, when not in use, filled with sterile 0.9 % NaCl containing 1,000 units of heparin sodium (Boots) per ml.

Introduction of catheters into the jugular vein. Catheters were introduced into the jugular vein under aseptic conditions with the aid of local lignocaine (Xylocaine, Astra) skin anaesthesia on the day preceding the absorption experiments. A polythene catheter (Sterivac No. 2, Allen and Handburys) was threaded through the lumen of a hypodermic needle which had previously been introduced into the jugular vein. The catheter was in turn anchored to the skin with two or three nylon monofilament sutures after first withdrawing the hypodermic needle from the vein.

#### Chemical Methods

A number of analytical procedures for the determination of VFAs and glucose in biological fluids are available. The methods used in these experiments were selected on the basis of their accuracy, specificity and simplicity. Their reliability was confirmed by repeatability and addition experiments (tables 3, 4, 5, 6, 7 and 8).

Blood was collected in 25 ml screw-capped glass bottles each of which

contained 500 i.u. of heparin sodium (Boots). The blood was deproteinized, for both glucose and VFA determinations, within 10 minutes of sampling in order to reduce the effects of post-sampling enzymic activity.

A plastic wash-bottle was used to obtain samples of rumen contents. Rumen liquor was aspirated and its pH determined within 2 minutes of sampling after first filtering it through two layers of cheese cloth. A Radiometer pH meter (Type PHM 23c) was used. Samples were retained in 25 ml screw-capped bottles for VFA analysis.

The sample bottles containing the rumen liquor and the protein-free filtrates of blood were stored at a temperature of 4°C. until the following day, when they were analysed. All laboratory glassware used in the analysis of these samples was previously washed in detergent (Delak No. 2, Shell), rinsed in hot tap water and dried at a temperature of 150°C. in an electric oven.

Blood volatile fatty acids (tables 3 and 4). Annison's (1954) method for the determination of blood VFAs was used. The following two modifications were however made:-

1. The neutralized protein-free filtrates were concentrated at a temperature of 80°C. in a drying oven instead of under vacuum. Concentration under vacuum was impractical at the time the samples were analysed. The use of a drying oven made it possible to concentrate a number of samples simultaneously. Loss of VFAs was not encountered since the potassium salts of these acids do not decompose or sublime at 80°C. (Handbook of Chemistry and Physics, 42nd Edition, 1960).
2. The steam distillates were titrated in the presence of a mixed

indicator containing Cresol Red and Thymol Blue (The Merck Index of Chemicals and Drugs, 1960) instead of using phenolphthalein. The mixed indicator has a sharper end-point than phenolphthalein at the transition pH of 8.3.

Results of repeatability and addition experiments are presented in tables 3 and 4 respectively.

TABLE 3

Blood volatile fatty acid determination repeatability experiment. Results of eight volatile fatty acid analyses on the same sample of bovine blood.

Sample	VFA concentration m-moles/lit	
1	1.09	
2	1.02	
3	1.08	<u>Mean:</u> 1.09 m-moles/lit
4	1.17	
5	1.11	
6	1.05	<u>Standard Deviation:</u> 0.0436 m-moles/lit
7	1.09	
8	1.09	

TABLE 4

Blood volatile fatty acid determination addition experiment. Results of eight volatile fatty acid analyses on bovine blood to which known quantities of acetic acid were added.

Sample	Determined VFA concentration m-moles/lit	Calculated VFA concentration m-moles/lit	$\frac{\text{Determined}}{\text{Calculated}} \times 100$	
1	1.09			
2	1.30	1.34	97.01	
3	1.70	1.60	106.25	
4	1.82	1.85	98.39	<u>Mean:</u> 99.71 %
5	2.12	2.10	100.95	
6	2.36	2.36	100.00	
7	2.59	2.61	99.23	<u>Range:</u>
8	2.75	2.86	96.15	96.15-106.25 %
9	3.11	3.12	99.68	

Blood glucose (tables 5 and 6). The Nelson-Somogyi method for the determination of blood glucose (Nelson, 1944; Somogyi, 1952) was used. Results of repeatability and addition experiments are presented in tables 5 and 6 respectively.

TABLE 5

Blood glucose determination repeatability experiment. Results of ten glucose analyses on the same sample of bovine blood.

Sample	Glucose concentration mg/100 ml	
1	64.46	
2	63.85	
3	62.65	
4	63.45	<u>Mean:</u> 63.89 mg/100 ml
5	65.66	
6	64.66	
7	63.45	<u>Standard Deviation:</u> 0.92 mg/100 ml
8	63.45	
9	62.85	
10	64.46	

TABLE 6

Blood glucose determination addition experiment. Results of five glucose analyses on bovine blood to which known quantities of glucose were added.

Sample	Determined glucose concentration mg/100 ml	Calculated glucose concentration mg/100 ml	$\frac{\text{Determined}}{\text{Calculated}} \times 100$	
1	46.96	-	-	
2	56.78	56.40	100.67	<u>Mean:</u> 101.46 %
3	66.13	65.65	100.73	
4	76.40	74.72	102.25	
5	84.58	83.61	101.16	<u>Range:</u>
6	94.63	92.34	102.48	100.67-102.48 %

Rumen liquor volatile fatty acids (tables 7 and 8). Johns' (1955) method for the determination of rumen liquor VFAs was used. Results of repeatability and addition experiments are presented in tables 7 and 8 respectively.

TABLE 7

Rumen liquor volatile fatty acid determination repeatability experiment. Results of ten volatile fatty acid analyses on the same sample of bovine rumen liquor.

Sample	VFA concentration m-moles/lit	
1	129.95	
2	130.56	
3	130.46	<u>Mean:</u> 130.34 m-moles/lit
4	130.87	
5	129.95	
6	129.64	
7	130.66	<u>Standard Deviation:</u> 0.39 m-moles/lit
8	130.66	
9	130.25	
10	130.36	

TABLE 8

Rumen liquor volatile fatty acid determination addition experiment. Results of ten volatile fatty acid analyses on bovine rumen liquor to which known quantities of volatile fatty acids were added.

Sample	Determined VFA concentration m-moles/lit	Calculated VFA concentration m-moles/lit	<u>Determined</u> <u>Calculated</u> x100	
1	85.64	-	-	
2	90.65	89.61	101.16	
3	95.30	93.51	101.91	
4	99.32	97.33	102.04	<u>Mean:</u> 101.37 %
5	101.92	101.08	100.83	
6	105.40	104.75	100.62	
7	110.05	108.36	101.56	<u>Range:</u> 100.62-102.04 %
8	113.36	111.90	101.30	
9	117.20	115.37	101.59	
10	120.60	118.78	101.53	
11	123.55	122.13	101.16	

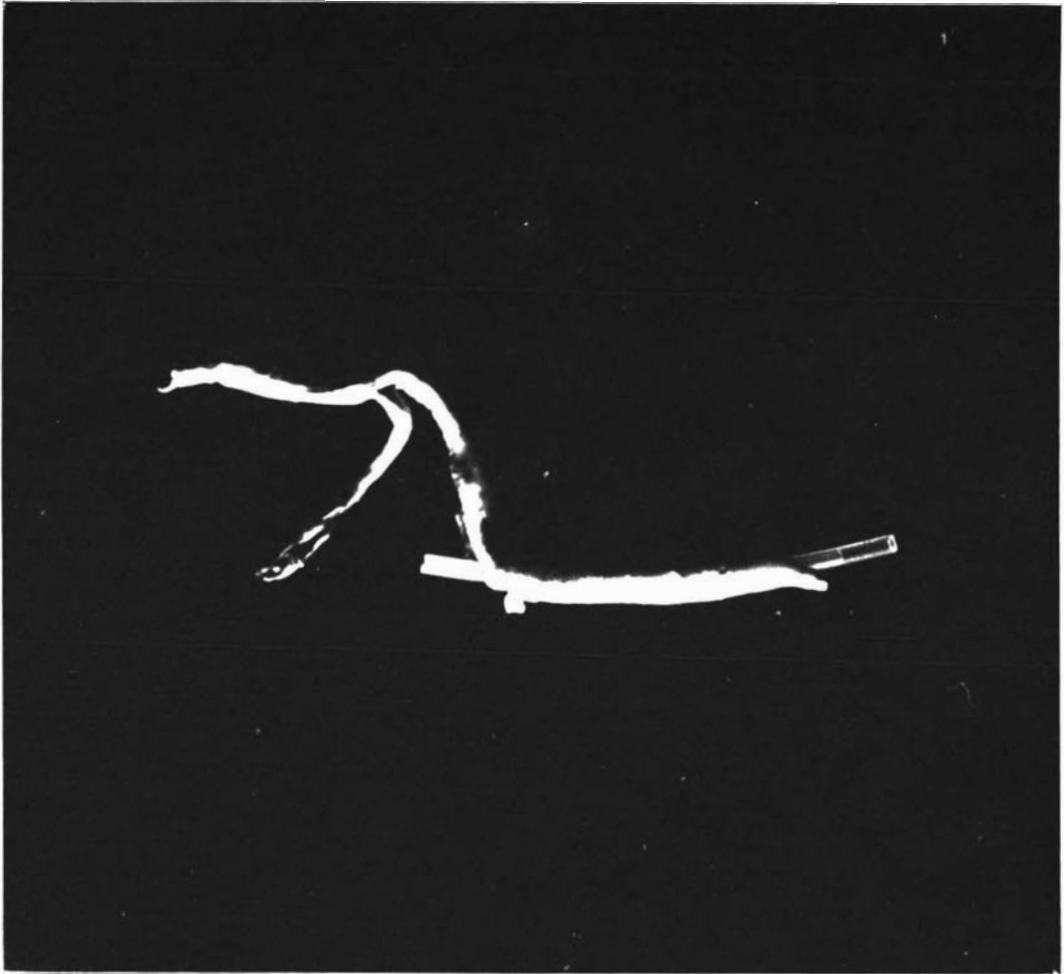


Fig. 1. Thrombus encapsulating the portal vein catheter.

## RESULTS

Portal vein catheters were successfully established in four young Friesian bull-calves. Three calves died during anaesthesia, and four others from post-operative haemorrhage. Portal vein catheters were introduced in another seven calves, but as a result of post-operative thrombophlebitis these catheters lost their patency within a few days after surgery and rendered the preparations useless for experimental purposes. On necropsy, a continuous fibrin sheath completely encapsulating the catheters from their point of entry into the venous circulation and extending well ahead of their tips was invariably found (fig. 1). The lumen of the catheters was usually free of clotted blood. Injection of solutions through the catheters into the portal circulation was always possible, but the thrombus apparently served as a valve which prevented the withdrawal of blood through them.

### Changes in the Volatile Fatty Acid Concentration and pH of Rumen Liquor Following Milk Feeding and Following Grazing (tables 9 and 10, fig. 2 and 3).

Observations on the changes in the VFA concentration and pH of rumen liquor following milk feeding were made in calves 2, 3 and 4, at the age of 36, 83 and 114 days respectively (table 2). The VFA concentration of rumen liquor at the end of fasting in calves 2, 3 and 4 was 22.16, 26.00 and 41.36 m-moles/lit respectively (table 9). The feeding of milk was followed by minor elevations of these values in calves 3 and 4, and by a more substantial increase in calf 2 (table 9, fig. 2).

The pH of rumen liquor at the end of fasting was 7.45, 7.65 and 7.35 in calves 2, 3 and 4 respectively (table 9, fig. 2). Milk feeding

TABLE 9

Changes in the volatile fatty acid concentration and pH of rumen liquor following milk feeding.

Calf	RUMEN LIQUOR VFA m-moles/lit						RUMEN LIQUOR pH					
	hr after feeding						hr after feeding					
	0	2	4	6	8	24	0	2	4	6	8	24
2	22.16	37.37	32.28	25.52	17.96	12.59	7.45	6.50	6.67	6.85	7.30	7.50
3	26.00	32.80	29.70	25.70	-	16.40	7.65	7.45	7.65	7.80	-	7.90
4	41.36	48.39	52.51	50.50	42.27	36.42	7.35	7.05	6.85	6.95	7.05	7.30

TABLE 10

Changes in the volatile fatty acid concentration and pH of the rumen liquor following grazing.

Calf	RUMEN LIQUOR VFA m-moles/lit						RUMEN LIQUOR pH					
	hr after feeding						hr after feeding					
	0	2	4	6	8	24	0	2	4	6	8	24
3	14.50	50.80	61.90	61.80	100.30	56.80	7.80	6.90	5.50	5.80	6.10	7.00
4	36.42	38.89	54.58	42.89	70.38	90.39	7.30	6.15	5.55	5.70	6.10	6.00

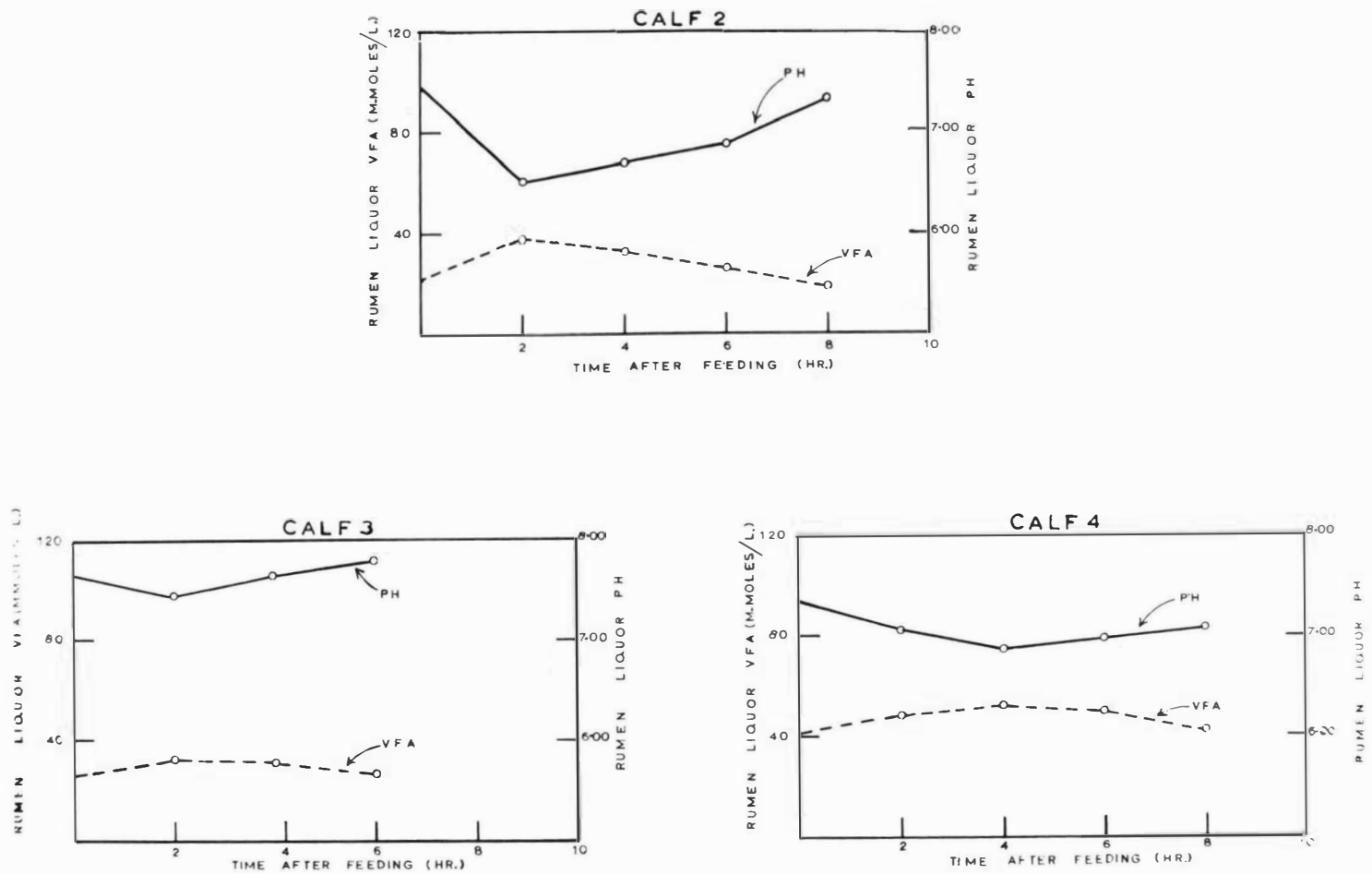


Fig. 2. Changes in the volatile fatty acid concentration and pH of rumen liquor following milk feeding.

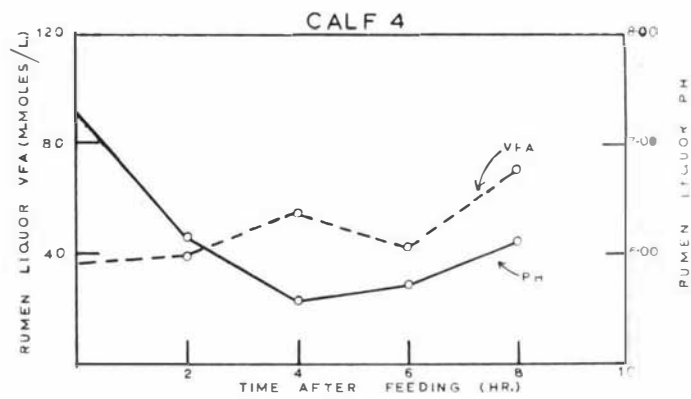
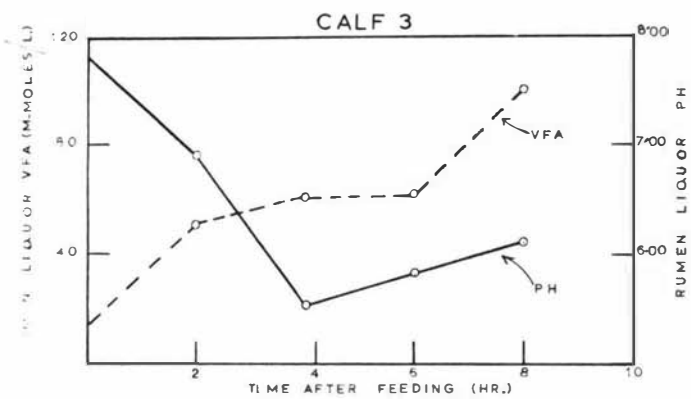


Fig. 3. Changes in the volatile fatty acid concentration and pH of rumen liquor following grazing.

was followed by an increase in the acidity of the rumen in calf 2, which reached a pH of 6.50 in 2 hours. There was only a slight lowering of the pH of the rumen in calves 3 and 4. In every case the highest concentration of VFAs that was detected in the rumen corresponded with the lowest pH recorded there (table 9, fig. 2).

Observations on the changes in the VFA concentration and pH of the rumen following grazing were made in calves 3 and 4, at the age of 78 and 115 days respectively (table 2). The VFA concentration of the rumen at the end of fasting was 14.50 m-moles/lit in calf 3, and 36.42 m-moles/lit in calf 4 (table 10). In calf 3, grazing resulted in an elevation of rumen VFAs to a level of 61.90 m-moles/lit within 4 hours after the calf commenced feeding (table 10). This initial rise was followed by a 2-hour period during which the VFA concentration of the rumen did not appear to change (table 10). The VFA concentration of the rumen then rose to a level of 100.30 m-moles/lit 8 hours after the beginning of grazing. A similar, though less pronounced and more delayed trend, was observed in calf 4, in which the VFAs rose from 36.42 m-moles/lit to 70.38 m-moles/lit during the first 8 hours after the beginning of grazing (table 10, fig. 3).

The rumen in both calves was alkaline at the end of the fasting period; a pH of 7.80 was recorded in calf 3 and 7.30 in calf 4. Grazing resulted in a marked decline in the pH of rumen liquor, which in both calves fell to approximately 5.50 within 4 hours after the beginning of feeding. In neither case did the highest concentration of VFAs recorded in the rumen correspond with the lowest pH detected there (table 10, fig. 3).

TABLE 11

Changes in volatile fatty acid and glucose concentrations of portal blood following milk feeding.

Calf	P O R T A L V F A m-moles/lit						P O R T A L G L U C O S E mg/100 ml					
	hr after feeding						hr after feeding					
	0	2	4	6	8	24	0	2	4	6	8	24
1*	0.62	0.94	0.87	-	-	-	62.61	154.28	73.29	-	-	-
2	0.58	0.89	-	0.69	0.79	0.60	59.84	139.96	108.40	75.20	81.20	65.70
3	0.53	0.84	1.58	0.67	1.00	0.72	38.72	133.06	136.98	106.20	71.69	50.83
4	0.91	1.30	0.91	0.94	0.88	0.73	40.59	91.84	72.80	70.50	50.42	44.14

\*Sampled at 1, 3, 7 and 10 hr after feeding, see fig. 2.

VFA portal concentrations at 1, 3, 7 and 10 hr were 0.84, 0.95, 0.75 and 0.67 m-moles/lit.

Glucose concentrations at 1, 3, 7 and 10 hr were 131.84, 146.79, 64.53 and 61.54 mg/100 ml.

TABLE 12

Changes in volatile fatty acid and glucose concentrations of portal blood following grazing.

Calf	P O R T A L V F A m-moles/lit						P O R T A L G L U C O S E mg/100 ml					
	hr after feeding						hr after feeding					
	0	2	4	6	8	24	0	2	4	6	8	24
3	0.91	-	2.50	1.79	2.23	1.27	47.01	51.71	62.39	70.94	69.44	60.26
4	0.73	1.08	1.70	1.14	1.52	1.18	44.14	45.87	50.83	46.90	52.48	47.31

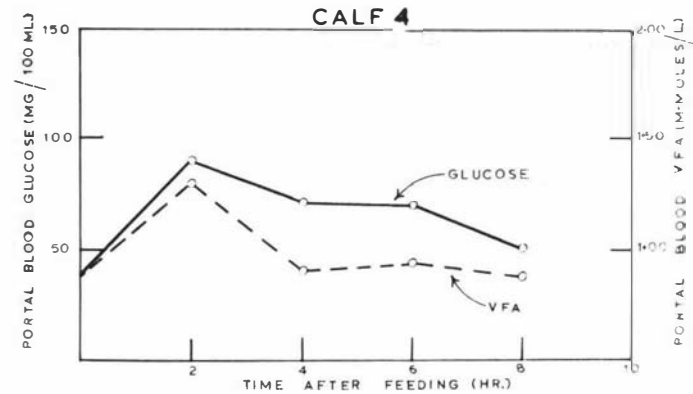
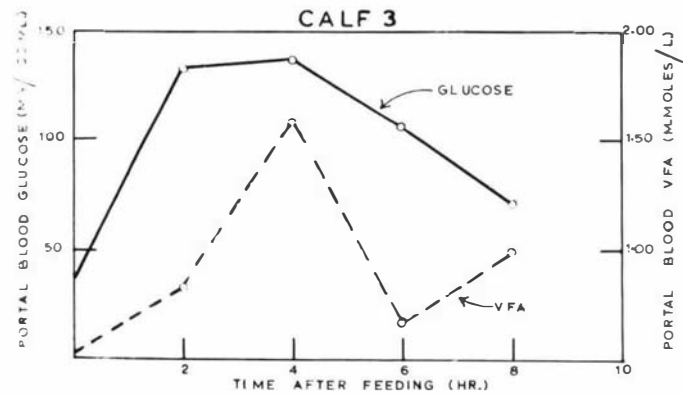
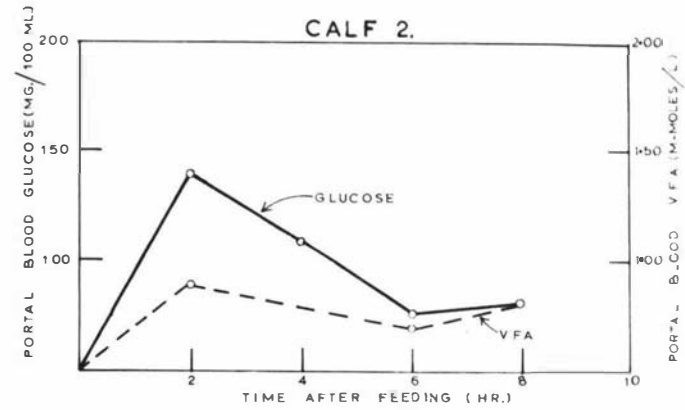
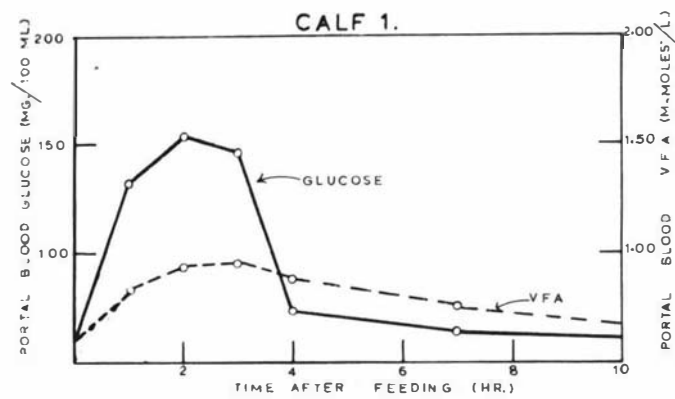


Fig. 4. Changes in volatile fatty acid and glucose concentrations of portal blood following milk feeding.

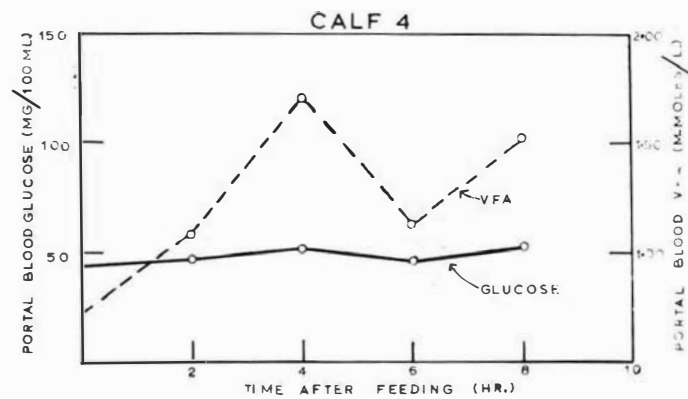
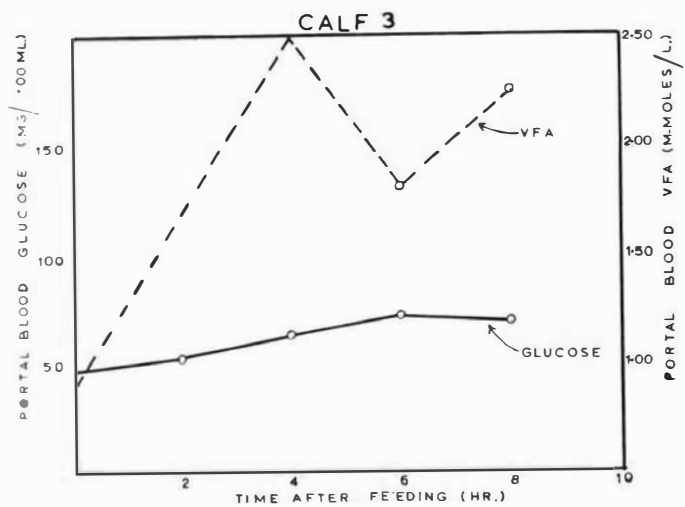


Fig. 5. Changes in volatile fatty acid and glucose concentrations of portal blood following grazing.

Changes in the Volatile Fatty Acid and Glucose Concentrations of Portal Blood Following Milk Feeding and Following Grazing (tables 11 and 12, fig. 4 and 5).

The concentration of portal blood glucose at the end of fasting was approximately 60 mg/100 ml of blood in the Milk Fed Calves (table 11) and approached 40 mg/100 ml of blood in the Pasture Fed Calves (table 12). Milk feeding resulted in marked elevations in the level of portal blood glucose in all four calves. A peak glucose concentration was reached 2 hours after feeding in calves 1, 2 and 4, and was apparently slightly delayed in the case of calf 3 (fig. 4). This peak concentration ranged from approximately 140-150 mg/100 ml of blood in calves 1, 2 and 3, and reached a level of 91.84 mg/100 ml of blood in calf 4. Following the initial rise of portal blood glucose, a precipitous decline to the fasting level was noted in calf 1. A progressive and slower return of portal blood glucose to its fasting concentration with advancing age following milk feeding was observed in the other three calves (fig. 4).

Although the VFA concentration of portal blood at the end of fasting in the Milk Fed Calves was in the order of 0.60 m-moles/lit of blood, that of the Pasture Fed Calves ranged from 0.53-0.91 m-moles/lit of blood (tables 11 and 12). Portal blood VFAs rose to a level of close to 0.90 m-moles/lit of blood 2 hours after the feeding of milk in calves 1 and 2, and to 1.30 m-moles/lit of blood during the same period in calf 4. A more pronounced and delayed elevation was encountered in calf 3.

Only minor changes in the glucose concentration of portal blood were observed following grazing in calf 4 (fig. 5). In contrast, however, a gradual increase of portal blood glucose from a level of 47.01 mg/100 ml

TABLE 13

Differences in glucose concentration of portal and jugular blood following milk feeding.

Calf	P O R T A L G L U C O S E mg/100 ml						J U G U L A R G L U C O S E mg/100 ml					
	hr after feeding						hr after feeding					
	0	2	4	6	8	24	0	2	4	6	8	24
4	40.59	91.84	72.80	70.50	50.42	44.14	42.68	83.05	67.36	62.97	49.37	41.42

TABLE 14

Differences in glucose concentration of portal and jugular blood following grazing.

Calf	P O R T A L G L U C O S E mg/100 ml						J U G U L A R G L U C O S E mg/100 ml					
	hr after feeding						hr after feeding					
	0	2	4	6	8	24	0	2	4	6	8	24
3	47.01	51.71	62.39	70.94	69.44	60.26	50.64	50.87	60.68	71.15	67.95	64.85
4	44.14	45.87	50.83	46.90	52.48	47.31	41.42	44.21	48.35	47.73	51.03	45.25

TABLE 15

Differences in the volatile fatty acid concentration of portal and jugular blood following milk feeding.

Calf	P O R T A L V F A m-moles/lit						J U G U L A R V F A m-moles/lit					
	hr after feeding						hr after feeding					
	0	2	4	6	8	24	0	2	4	6	8	24
4	0.91	1.30	0.91	0.94	0.88	0.73	0.60	1.09	1.33	0.86	0.86	0.77

TABLE 16

Differences in the volatile fatty acid concentration of portal and jugular blood following grazing.

Calf	P O R T A L V F A m-moles/lit						J U G U L A R V F A m-moles/lit					
	hr after feeding						hr after feeding					
	0	2	4	6	8	24	0	2	4	6	8	24
3	0.91	-	2.50	1.79	2.23	1.27	0.78	-	1.00	0.92	1.42	1.07
4	0.73	1.08	1.70	1.14	1.52	1.18	0.77	1.01	1.34	0.88	1.04	0.92

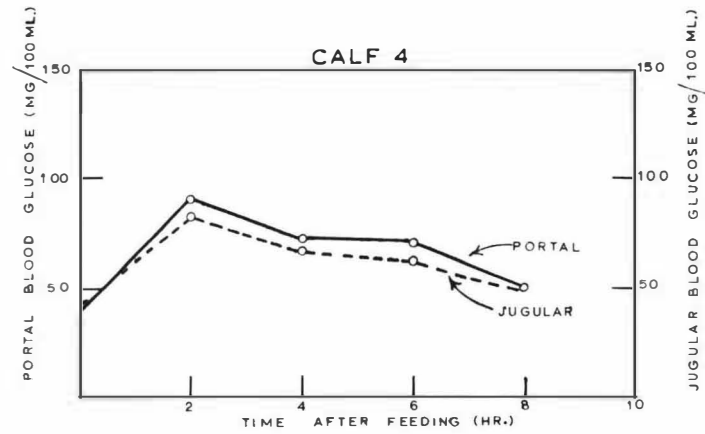


Fig. 6. Differences in glucose concentration of portal and jugular blood following milk feeding.

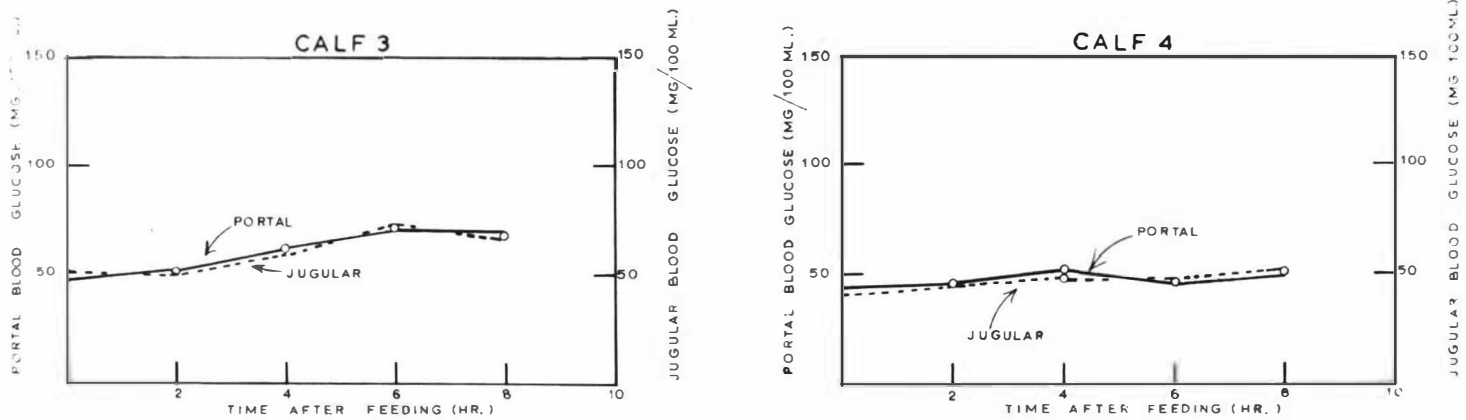


Fig. 7. Differences in glucose concentration of portal and jugular blood following grazing.

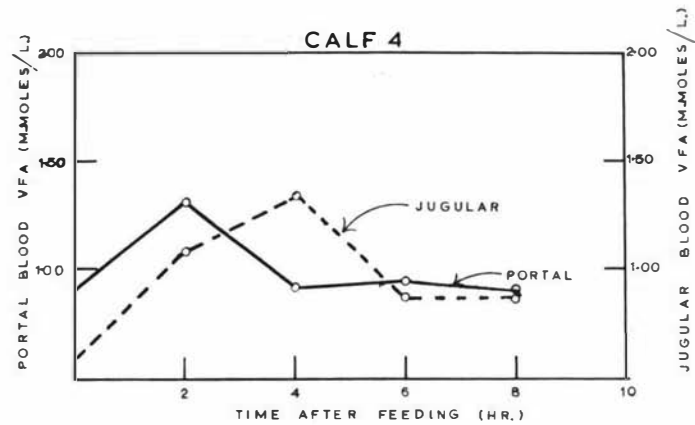


Fig. 8. Differences in the volatile fatty acid concentration of portal and jugular blood following milk feeding.

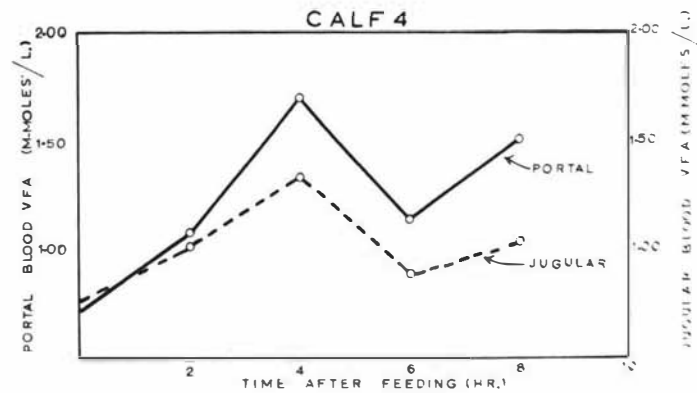
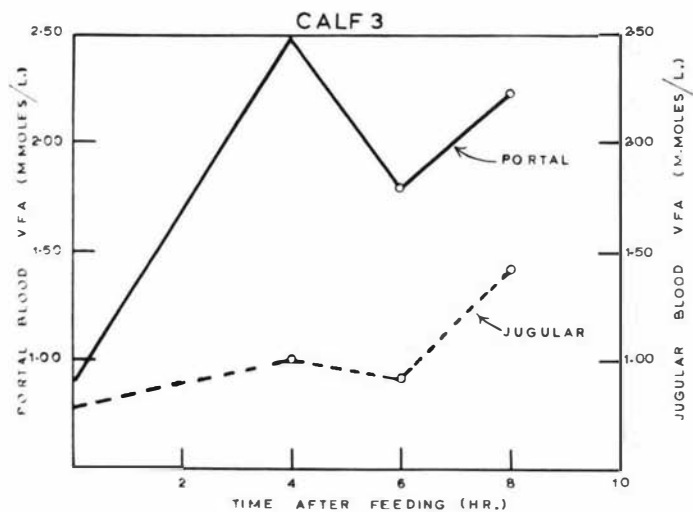


Fig. 9. Differences in the volatile fatty acid concentration of portal and jugular blood following grazing.

of blood at the end of fasting to a concentration of 70.94 mg/100 ml of blood 6 hours after the beginning of grazing was noted in calf 3 (fig. 5).

Similar changes in the levels of portal blood VFAs were noted in both Pasture Fed Calves following grazing. A primary peak VFA concentration was recorded 4 hours after the beginning of grazing, and a secondary peak after another 4 hours (fig. 5).

Differences in the Glucose and Volatile Fatty Acid Concentrations of Portal and Jugular Blood Following Milk Feeding and Following Grazing (tables 13, 14, 15 and 16, fig. 6, 7, 8 and 9).

Jugular blood samples following milk feeding were collected from calf 4 only, and after grazing from calves 3 and 4. Glucose concentrations of simultaneously collected jugular and portal vein blood samples were invariably within a few milligrams of each other, irrespective of whether milk or pasture was consumed (tables 13 and 14, fig. 6 and 7).

The VFA concentration difference of portal and jugular vein blood in calves 3 and 4, at the end of fasting and before grazing, was small (table 16, fig. 9). A marked post-prandial increase in the magnitude of the VFA concentration difference in favour of the portal vein was noted in both calves, and was more pronounced in the case of calf 3 (table 16, fig. 9). Although the portal and jugular blood VFAs rose at similar rates following milk consumption in calf 4 (table 15, fig. 8) the highest VFA concentration was recorded within 2 hours after the beginning of grazing in the portal blood of this calf, and within 4 hours in the jugular blood (table 16, fig. 9).

## DISCUSSION

The technical difficulties encountered in the establishment and maintenance of portal vein catheters precluded more and better controlled trials. Other reports of experiments involving the chronic catheterization of the portal vein in ruminants have in common the difficulties encountered in maintaining the patency of these catheters. Although Conner and Fries (1960), Moodie, Walker and Hutton (1963), Parker, Simesen and Luick (1963), have reported occasional cases where portal vein catheters have remained functional for periods exceeding 6-10 weeks, Annison et al. (1957), Conner and Fries (1960), Bensadoun and Reid (1962) and Waldern et al. (1963) indicate that portal vein catheters can normally be expected to retain their patency for far shorter periods, usually for not more than 2-4 weeks after their surgical insertion.

The following observations on the development of post-operative thrombophlebitis were made:-

1. The condition developed sooner in younger than in older calves.
2. Thrombus formation started at the point of entry of the catheter into the venous circulation.
3. The thrombus increased in size with time until it completely invested the catheter, and eventually extended downstream beyond the tip of the catheter.
4. It was impossible to displace the valve-forming thrombus by passage of a stilette down the catheter.
5. More acute reactions occurred with silicone treated polyvinyl catheters than with either polythene or Teflon tubing.
6. Prothrombinopenic (Marevan, Evans Medical) and fibrinolytic (Varizyme, American Cyanamid) medication appeared to retard but

not to prevent the development of post-operative thrombophlebitis.

7. The problem of thrombophlebitis was not encountered in calves with polythene catheters inserted in the jugular vein.

Changes in the Volatile Fatty Acid Concentration and pH of Rumens Liquor Following Milk Feeding and Following Grazing (tables 9 and 10, figs. 2 and 3).

Ingested milk in intact young ruminants normally short-circuits the rumen and passes directly into the abomasum (Watson, 1944; Comline and Titchen, 1961). Milk may however inadvertently enter into the rumen as a result of regurgitation from the abomasum, incomplete closure of the oesophageal groove, and inhibition of the oesophageal groove reflex (Comline and Titchen, 1961). Any milk that enters the rumen of young milk-fed calves undergoes a predominantly lactic acid-producing fermentation (Mann and Oxford, 1955; Mackay and Oxford, 1954; Bryant, Small, Bouma and Robinson, 1958). Such a fermentation should in turn be expected to result in a marked depression of rumen pH if much milk enters the organ.

Normally the oesophageal groove serves to direct milk into the abomasum (Watson, 1944; Comline and Titchen, 1961), whereas solid feeds enter directly into the rumen (Annison and Lewis, 1959). These findings account for the post-prandial changes in the VFA concentration and pH of rumen liquor in which greater increases in acidity and VFA levels were recorded following grazing (table 10, fig. 3) than after milk feeding (table 9, fig. 2).

Calf 2 was fed proportionately more milk on a body weight basis than were calves 3 and 4 (table 2). Greater post-prandial changes in the VFA concentration and pH of the rumen were encountered in this calf than in the

other two calves (table 9, fig. 2). This may have been caused by the entry of milk into the rumen and the fermentation of this milk by lactic acid-producing micro-organisms. Both Warner et al. (1956) and Tamate et al. (1962) have demonstrated that the abomasal capacity in milk-fed calves of a similar age to calf 2 is considerably less than 4 lit, the volume of milk fed to this animal. Comline and Titchen (1951) have also shown that abomasal distention, at least in decerebrate calves, inhibits the reflex closure of the oesophageal groove and causes fluids to enter directly into the rumen.

An alkaline rumen was noted at the end of the fasting period in all of the calves irrespective of their age or diet (tables 9 and 10). This observation is in agreement with the recognized effects of fasting on the pH of rumen liquor, namely "pH rises to 7 or 8 in 48 hours" (Annison and Lewis, 1959).

An inverse relationship between the VFA concentration and pH of the rumen has been reported in mature ruminants by Baloh and Rowland (1957), Briggs, Hogan and Reid (1957), Davey (1964). Although the rapid decline of rumen pH to 5.5 within 4 hours after the beginning of grazing in calves 3 and 4 is in agreement with their findings, the simultaneous increase in both the pH and VFA concentration of the rumen for the next 4 hours (4-8 hours after the beginning of grazing) is not (table 10, fig. 3). This observation indicates that the pH of the rumen in young calves is not wholly dependent on the VFA concentration of rumen liquor. It suggests either the presence of an active lactic acid fermentation, or the absence of an adequate buffering capacity within the rumen of young calves. Evidence that both mechanisms may be involved is presented in the work of Bryant et al. (1958) and Eadie, Hobson and Mann (1959), who demonstrated a

persistence of lactic acid-producing micro-organisms in the rumen up to the age of 15 weeks, and by the experiments of Kay (1958) in calves, and Wilson and Tribe (1961) in lambs, who noted a 7-8 week period of post-natal maturation of the parotid glands in these animals.

Volatile fatty acids are produced in the rumen by the microbial fermentation of solid feeds (Annison and Lewis, 1959; Barnett and Reid, 1961). The microbiological population of the rumen in 1-6 week-old milk-fed calves is largely composed of lactic acid-producing organisms (Bryant et al., 1958). In his review Hobson (Kay and Hobson, 1963) states that the change from a predominantly lactic acid-producing flora to a microbial population characteristic of the adult rumen is largely a function of the solid feeds consumed. The experiments of Wardrop and Coombe (1961) in lambs and Godfrey (1961) and Stewart (1962) in calves indicate that a 2-6 week period of time on pasture is required before mature VFA concentrations can be demonstrated in the rumen. Although grazing in calf 3 was followed by increases in the VFA concentration of the rumen similar to those found in mature ruminants (table 10), fasting resulted in a precipitous decline in the level of ruminal VFAs (tables 9 and 10). This observation suggests an immature microbial population in the rumen. It further indicates that the demonstration of mature VFA concentrations in the rumen may not constitute sufficient evidence to indicate that a microbial population similar to that found in adult ruminants has been established.

Changes in the Volatile Fatty Acid and Glucose Concentrations of Portal Blood Following Milk Feeding and Following Grazing (tables 11 and 12, fig. 4 and 5).

A gradual post-natal decline in the fasting level of circulating glucose in the peripheral blood of calves from levels of 90-100 mg/100 ml at birth to 60-65 mg/100 ml at 6-7 weeks of age has been reported by a number of workers (McCandless and Dye, 1950; Martin et al., 1959; Warner et al., 1959). The limited observations made in the present study indicate a similar decrease in the fasting level of portal blood glucose with advancing age (tables 11 and 12).

McCandless and Dye (1950) and Warner et al. (1959) also demonstrated a decrease in the tolerance of calves to intravenously imposed loads of glucose during the first 3-4 months of post-natal life. The progressive slowing in the return of portal blood glucose to its fasting concentration with advancing age following the feeding of milk (table 11, fig. 4) seems to parallel the post-natal decline in the tolerance of bovines to intravenously administered glucose reported by McCandless and Dye (1950) and Warner et al. (1959).

Fasting concentrations of portal blood VFAs differed considerably in the same calf on separate occasions which were only 1-5 days apart (e.g. calves 3 and 4, tables 11 and 12). The experiments did not reveal an increase in the fasting level of portal blood VFAs with advancing age or with free and continuous access to pasture (tables 13 and 14). This observation conforms with that made by Dinda (cited by Preston, 1963) on jugular blood in early-weaned calves; it is not in agreement with the findings of either Martin et al. (1959) who noted low and unchanging

starvation levels of jugular blood VFAs with advancing age, or with those of McCarthy and Kesler (1956), who demonstrated an increase in these levels with age.

The post-prandial increase in the VFA concentration of portal blood following milk feeding (table 11, fig. 4) in calves in which the oesophageal groove appeared to be functioning has not been reported previously. This observation suggests the existence of extra-ruminal sources of circulating VFAs. Caecal fermentation has been shown to account for less than 5 % of the total VFAs produced in the alimentary tract of mature ruminants (Hungate, Phillips, McGregor, Hungate and Buechner, 1959). Volatile fatty acid production in the caecum of young ruminants has not been investigated to date. Accordingly one can only speculate on the caecal contributions of VFAs to the portal circulation in calves. It is unlikely, however, that this source of VFAs was wholly responsible for the marked concentration changes observed in the portal blood following milk feeding (table 11, fig. 4). The similar glucose and VFA concentration changes of portal blood (table 11, fig. 4) suggest another likely source of circulating VFAs, namely the endogenous metabolism of absorbed dietary glucose. Irrespective of the origin of these VFAs - caecal fermentation or endogenous metabolism - the experiments indicate that VFA absorption studies based on the analysis of blood should not be performed in young ruminants following the feeding of milk. A further point which must be kept in mind is that roughage consumption in milk-fed calves may fail to demonstrate VFA absorption, not because of an inability to absorb VFAs, but because a microbial population capable of fermenting solid feeds would not be established under such a feeding system. Under these circumstances the capacity to absorb VFAs is better tested by the introduction of preformed salts of these acids into the rumen.

A comparison can be made between the VFA concentration changes of portal blood following grazing in calf 4, and those reported in mature sheep by Schambye and Phillipson (1949), Schambye (1951 a & b), and Annison et al. (1957), and in calves several months older than calf 4 by Waldern et al. (1963). In terms of the VFA concentration changes of the rumen and of portal blood, this 4-month-old pasture-reared calf was equivalent to a mature ruminant.

The absence of a post-prandial hyperglycaemia following grazing in calf 4 (table 14, fig. 7) is in agreement with observations made in mature ruminants (Schambye and Phillipson, 1949; Schambye, 1951 a, b & c; Annison et al., 1957), and can be attributed to the microbial fermentation of soluble carbohydrates in the rumen (Annison and Lewis, 1959). Although the pH and VFA concentration changes of the rumen following grazing in calf 3 (table 14, fig. 7) are similar to those observed in adult ruminants (Schambye and Phillipson, 1949; Schambye, 1951 a & b; Annison and Lewis, 1957), the post-prandial hyperglycaemia noted in this calf is not. Glucose has been shown to be absorbed from the rumen of mature animals following acute starvation and following the removal of rumen contents (Dougherty, Allen, Burroughs, Jacobson and McGilliard, 1956) and from the intestines of early-weaned concentrate-fed calves, by the intestinal hydrolysis of dietary starch which escapes rumen fermentation (Preston and Ndumbe, 1961). Regardless of whether the post-prandial hyperglycaemia observed in calf 3 following grazing (table 14, fig. 7) was caused by glucose absorption from the rumen or from the intestines, it nevertheless indicates the presence of an "immature" microbial population within the rumen. This observation further suggests that the demonstration of high VFA concentrations in the rumen of pasture-reared calves by the third or fourth week of age (Godfrey 1961; Stewart,

1962) need not by itself necessarily constitute sufficient evidence to indicate the establishment within the rumen of a microbial population that is characteristic of the mature ruminant.

Differences in the Glucose and Volatile Fatty Acid Concentrations of Portal and Jugular Blood Following Milk Feeding and Following Grazing (tables 13, 14, 15 and 16, fig. 6, 7, 8 and 9).

The glucose concentrations of simultaneously collected portal and jugular blood samples were found to be within a few milligrams of one another, irrespective of whether milk or pasture was fed (tables 13 and 14, fig. 6 and 7). This is in agreement with the observations of Schambye and Phillipson (1949), Schambye (1951 a and b) and Annison et al. (1957) in mature sheep fed solid test diets. Although the levels of portal blood glucose were usually higher than the corresponding jugular concentrations, there were occasions when the opposite was the case. These occasions were encountered at the end of the fasting periods (0 hour samples, calf 3, table 14, fig. 7; calf 4, table 13, fig. 6), and at the times when high concentrations of VFAs were recorded within the rumen and in the portal blood (tables 13 and 14, fig. 6 and 7). The inherent limitations of the analytical procedure (tables 5 and 6) may have been partly or wholly responsible for these seeming discrepancies. Another likely explanation is that gluconeogenesis, from body reserves during starvation and from the endogenous metabolism of absorbed dietary propionate following grazing, is accompanied by the mobilization of glucose, which in turn leads to the presence of higher levels of glucose in the peripheral than in the portal circulation.

The substantial VFA concentration differences between simultaneously collected portal and jugular blood samples following grazing (table 16, fig. 9) indicate the rapid removal of absorbed VFAs from the circulation, and are in agreement with the observations made in adult sheep by Schambye and Phillipson (1949), Schambye (1951 a and b) and Annison et al. (1957). They further show that absorbed VFAs are more rapidly cleared from the circulation than has been observed with absorbed glucose.

The VFA concentration differences between simultaneously collected portal and jugular blood samples following milk feeding (table 15, fig. 8), on the other hand, are much less pronounced. This apparent lowered clearance of circulating VFAs may be considered as further evidence in support of the suggestion that the endogenous metabolism of absorbed dietary glucose may give rise to an increased level of circulating VFAs.

#### SUMMARY

1. The effects of advancing age and free and continuous access to pasture on VFA absorption from the rumen were investigated in young conscious calves by the analysis of portal blood.
2. Portal vein catheters lost their patency within 2 weeks after their surgical insertion as a result of post-operative thrombophlebitis. Prothrombinopenic and fibrinolytic medication delayed but did not prevent the development of thrombophlebitis.
3. Long-term comparative studies with calves reared on different dietary regimes were precluded. Experiments in four Friesian bull-calves of various ages and in different stages of rumen development were therefore performed.

4. The experiments indicated:-

- (a) A decline in the starvation level of portal blood glucose with advancing age in four separate calves.
- (b) A reduction in the tolerance of calves to glucose with advancing age.
- (c) The existence of extra-ruminal sources of circulating VFAs.
- (d) The rapid removal of absorbed VFAs from the circulation.
- (e) An "immature" microbial population in the rumen of a 10-week-old weaned pasture-reared calf.

5. Fasting levels of circulating VFAs characteristic of the age or diet of the calves were not demonstrated.

6. The experiments suggested:-

- (a) The need for investigating the metabolism of glucose in calves.
- (b) The possible value of introducing preformed salts of the VFAs into the rumen in future absorption studies.

## CHAPTER II

### GLUCOSE: A SOURCE OF STEAM VOLATILE FATTY ACIDS IN THE BLOOD OF CALVES

A post-prandial increase in the VFA concentration of portal blood was demonstrated in four Friesian bull-calves following milk feeding (Chapter I, table 11, fig. 4). This increase appeared to be independent of ruminal fermentation (table 9, fig. 2). Since the VFA and glucose concentration changes of portal blood followed a similar time-course (table 11, fig. 4), it was suggested that the metabolism of absorbed dietary glucose may lead to an increase in the level of circulating VFAs.

Three experimental approaches were adopted to determine the effects of an induced hyperglycaemia on the concentration of circulating VFAs. The level of glucose in the blood was enhanced by:

1. The feeding of skim milk supplemented with lactose (Experiment I).
2. The intravenous administration of glucose (Experiment II).
3. The intravenous administration of adrenaline (Experiment III).

## REVIEW OF LITERATURE

Martin et al. (1959) studied the post-prandial concentration changes of glucose and VFAs in the jugular blood of young calves fed different test diets. Although these workers demonstrated marked elevations in the levels of circulating glucose in jugular blood following milk feeding, they did not observe similar increases in the VFA concentrations.

No reference was found in the literature consulted of other attempts to investigate the problem in young calves. This paucity of information may have been caused by a preoccupation with the exogenous sources of circulating VFAs (Annison and Lewis, 1959; Barnett and Reid, 1961). Evidence has however accumulated over recent years to indicate both a close relationship between the metabolism of glucose and VFAs and the endogenous origin of some of the circulating VFAs.

Jarrett and Potter (1957) studied the tolerance of normal and diabetic sheep to imposed loads of intravenously injected acetate, propionate and butyrate. They reported grossly elevated levels of both glucose and VFAs in the jugular blood of depancreatized sheep but not in those made diabetic with alloxan. Reid (1958) undertook glucose and acetate tolerance tests in sheep fed on different diets and observed that the disappearance rates of glucose and acetate from the circulation were in the same direction and of a similar order of magnitude. Jarrett and Filsell (1961) conducted acetate tolerance tests in sheep and showed that the prior-intravenous administration of glucose led to a more rapid removal of acetate from the blood than in control experiments in which glucose was not given. On the basis of this observation they suggested that glucose may be important in aiding the "utilization" of acetate. In a short note Lindsay (1959 a) reported that

interference with glucose utilization in mature sheep, e.g. by the administration of insulin, adrenaline or alloxan, resulted in an increase in the level of circulating VFAs.

Endogenous VFAs were demonstrated in the blood of sheep by Annison (1954). His experiments showed that the VFA fraction of sheep blood contained 10-30 % formic acid. He was unable to detect this acid in the alimentary tract contents and suggested that the formate in peripheral blood was endogenous in origin. Further evidence for the endogenous contribution of VFAs to the circulation was presented in the radio-isotope studies of Annison and White (1962) in sheep and Lee and Williams (1962) in cattle. Both groups of workers estimated that approximately 25 % of the post-prandial acetate found in the blood was of endogenous origin.

## EXPERIMENTAL

### Experiment I. A Hyperglycaemia Induced by the Feeding of Skim Milk Supplemented with Lactose

#### Materials and Methods

One 36-day-old Friesian bull-calf which had been reared on a diet of milk only was used in this experiment (Calf 2, table 1). A rumen fistula and a portal vein catheter had been previously introduced into this calf as was described in Chapter I. The calf was fasted for a period of 24 hours and was then fed 4 lit of whole milk (4.5 % fat). Simultaneous samples of rumen liquor and portal blood were collected before and at 2-hour intervals after feeding. This blood was analysed for glucose and VFA concentrations by the methods described earlier (Chapter I). A similar procedure was

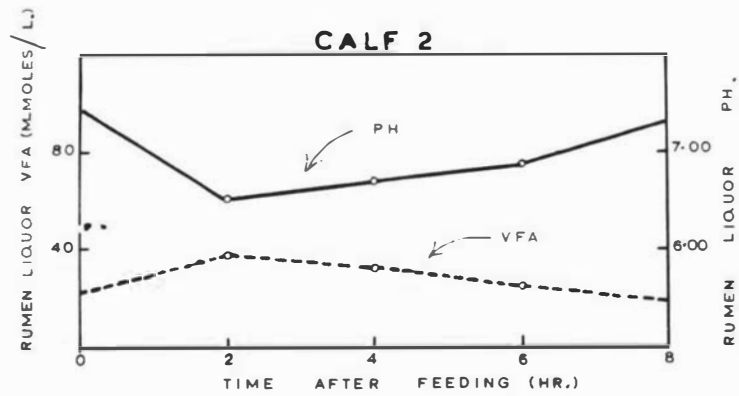


Fig. 10. Changes in the volatile fatty acid concentration and pH of rumen liquor following the feeding of whole milk.

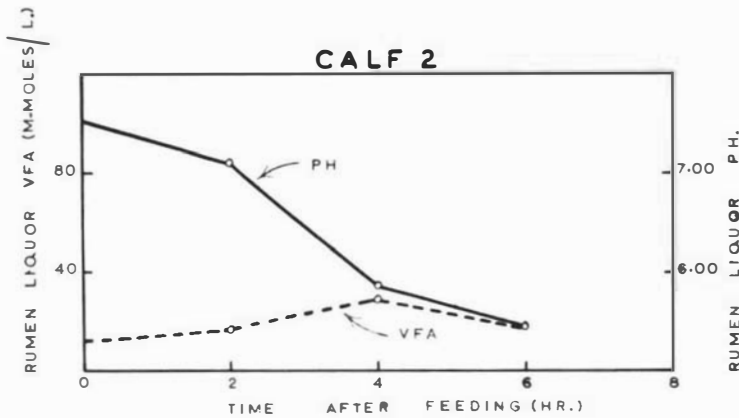


Fig. 11. Changes in the volatile fatty acid concentration and pH of rumen liquor following the feeding of skim milk supplemented with lactose.

followed on the next day except that instead of whole milk 4 lit of skim milk containing 10 % (w/v) of added lactose (May and Baker) were fed.

Results

Changes in the volatile fatty acid concentration and pH of rumen liquor following the feeding of whole milk and following the feeding of skim milk supplemented with lactose (table 17, fig. 10 and 11). Although only minor increases in the VFA concentration of the rumen were observed following the feeding of either whole milk or skim milk supplemented with lactose, a substantial decline in rumen pH was noted on both occasions (table 17, fig. 10 and 11). The pH of the rumen fell from 7.45-6.50 within 2 hours of whole milk feeding (table 17, fig. 10) and from 7.10-5.45 over the first 6 hours following the feeding of skim milk supplemented with lactose (table 17, fig. 11).

TABLE 17

Changes in the volatile fatty acid concentration and pH of rumen liquor following the feeding of whole milk and following the feeding of skim milk supplemented with lactose.

Test Diet	Time after feeding hr	R U M E N L I Q U O R	
		VFA m-moles/lit	pH
WHOLE MILK	0	22.16	7.45
	2	37.37	6.50
	4	32.28	6.67
	6	25.52	6.85
	8	17.96	7.30
	24	12.59	7.50
SKIM MILK + 10 % LACTOSE	0	12.59	7.50
	2	17.40	7.10
	4	28.93	5.81
	6	19.97	5.45
	8	-	-
	24	-	-

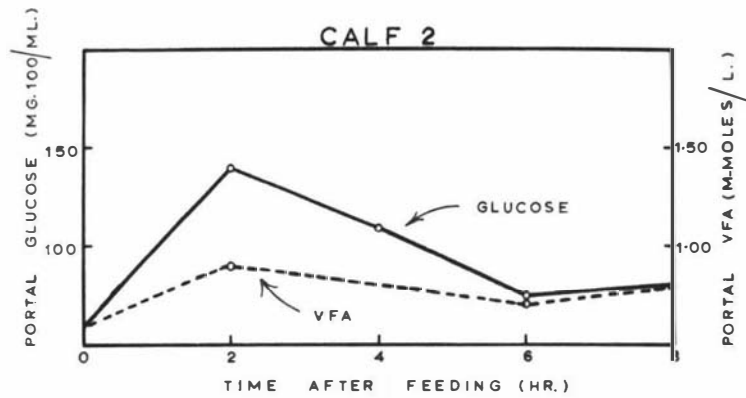


Fig. 12. Changes in the glucose and volatile fatty acid concentrations of portal blood following the feeding of whole milk.

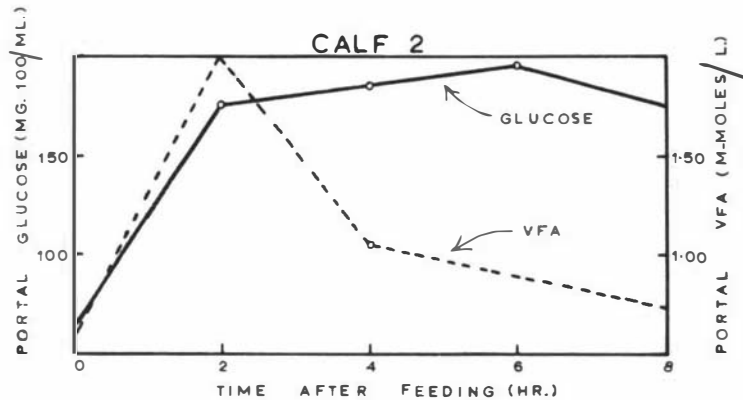


Fig. 13. Changes in the glucose and volatile fatty acid concentrations of portal blood following the feeding of skim milk supplemented with lactose.

Changes in the glucose and volatile fatty acid concentrations of portal blood following the feeding of whole milk and following the feeding of skim milk supplemented with lactose (table 18, fig. 12 and 13). A post-prandial increase in the glucose and VFA concentrations of portal blood was observed following the feeding of both test diets (table 18, fig. 12 and 13). Greater changes in the glucose and VFA levels of portal blood were recorded after the consumption of skim milk supplemented with lactose than when a similar volume of whole milk was fed (table 18, fig. 12 and 13).

TABLE 18

Changes in the glucose and volatile fatty acid concentrations of portal blood following the feeding of whole milk and following the feeding of skim milk supplemented with lactose.

Test Diet	Time after feeding hr	P O R T A L B L O O D	
		Glucose mg/100 ml	VFA m-moles/lit
WHOLE MILK	0	59.84	0.58
	2	139.96	0.89
	4	108.40	-
	6	75.20	0.69
	8	81.20	0.79
	24	65.70	0.60
SKIM MILK + 10 % LACTOSE	0	65.70	0.60
	2	175.00	2.02
	4	185.33	1.08
	6	195.87	-
	8	175.62	0.71
	24	84.11	0.63

The pre-feeding concentration of portal blood glucose rose from a level of 59.84 to 139.96 mg/100 ml during the first 2 hours after whole

milk feeding and then declined to a level of 75.20 mg/100 ml within 6 hours after feeding (table 18, fig. 12). In contrast the concentration of glucose in the portal blood rose from 65.70 to 175.00 mg/100 ml within 2 hours after the feeding of skim milk supplemented with lactose, and continued to rise over the next 4 hours so that a level of 195.87 mg/100 ml was detected 6 hours after feeding (table 18, fig. 13).

Portal blood VFAs rose from 0.58 to 0.89 m-moles/lit during the first 2 hours after whole milk feeding (table 18, fig. 12) and from 0.60 to 2.02 m-moles/lit over the same period following the feeding of skim milk supplemented with lactose (table 18, fig. 13). This high portal blood VFA concentration, unlike the hyperglycaemia, was not maintained; it declined precipitously to a level of 1.08 m-moles/lit 4 hours after feeding (table 18, fig. 13).

## Experiment II. A Hyperglycaemia Induced by the Intravenous Injection of Glucose

### Materials and Methods

Four bull-calves were used in this study. Details of their age, breed and weight are presented in table 19. Jugular vein catheters were introduced into these calves on the day preceding the experiments in a manner similar to that described in Chapter I. The calves were fasted for 24 hours off milk and 48 hours off pasture. A hyperglycaemia was induced at the end of the fasting period by the intravenous injection of a 50 % (w/v) aqueous solution of D-glucose (autoclaved at 115°C. for 30 minutes) into the jugular vein; as was the practice adopted by Reid (1958) in sheep. Two glucose dose rates were used, namely: 1 gm/kg of body weight

TABLE 19

Details concerning the breed, age, weight and age at weaning of the calves that were used in the intravenous glucose infusion experiments.

Calf	Breed	Weaned days	Age days	Weight kg	Quantity of glucose administered gm	Starvation off Milk hr	Pasture hr
5	Friesian	-	57	55	55	24	48
6	Friesian	-	57	59	59	24	48
7	Jersey	70	102	68	34	-	24
8	Jersey	69	101	60	30	-	24

TABLE 20

Changes in the glucose and volatile fatty acid concentrations of jugular blood following the intravenous injection of 50 % (w/v) D-glucose and following the intravenous injection of 0.9 % NaCl.

Calf	Glucose administered gm	Vol. of 0.9 % NaCl administered ml	JUGULAR GLUCOSE mg/100 ml					JUGULAR VFA m-moles/lit				
			0 min	15 min	60 min	120 min	180 min	0 min	15 min	60 min	120 min	180 min
5	55	-	51.83	239.84	145.93	81.71	38.82	1.33	4.86	4.27	1.95	0.94
6	59	-	61.75	301.28	200.43	136.75	92.31	1.40	6.19	4.24	2.97	2.57
7	34	-	45.09	204.60	131.29	82.82	51.53	2.20	3.61	3.82	2.80	2.38
8	30	-	41.41	178.83	120.25	80.98	54.29	1.90	4.27	3.60	2.27	2.09
7	-	68	47.51	47.83	47.20	43.48	39.48	2.68	2.42	2.26	2.23	2.53
8	-	60	35.09	40.37	40.99	40.37	37.58	2.01	2.05	2.27	2.14	2.14

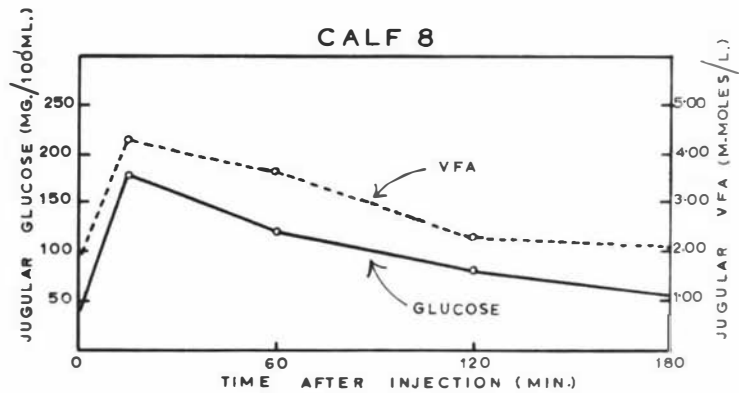
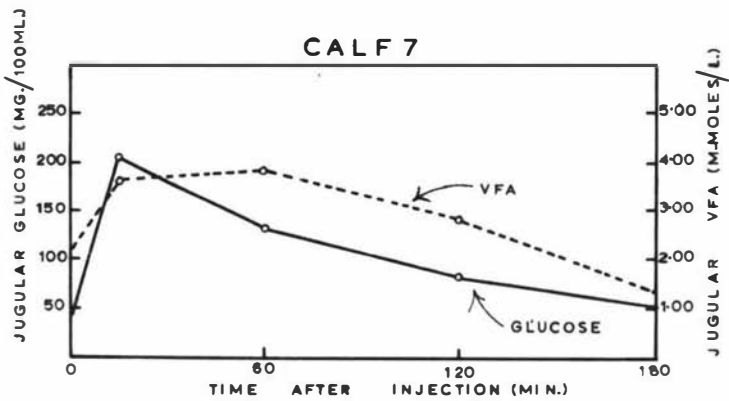
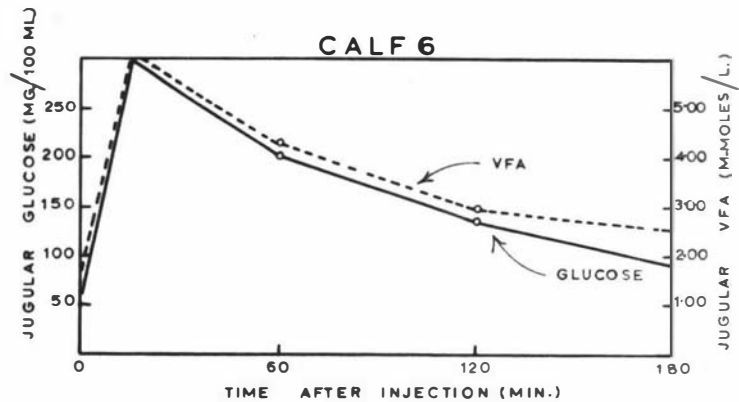
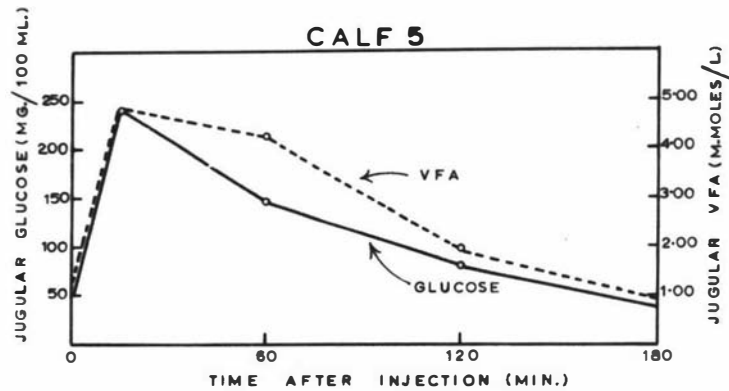


Fig. 14. Changes in the glucose and volatile fatty acid concentrations of jugular blood following the intravenous injection of 50% (w/v) D-glucose.

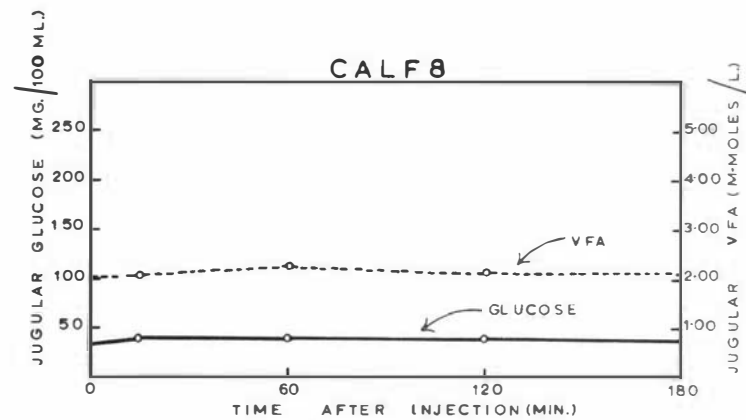
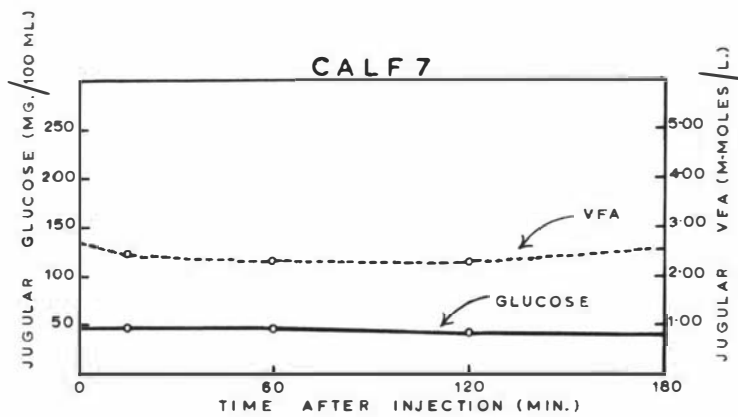


Fig. 15. Changes in the glucose and volatile fatty acid concentrations of jugular blood following the intravenous injection of 0.9 % NaCl.

in calves 5 and 6, and 0.5 gm/kg of body weight in calves 7 and 8 (table 19). Irrespective of its volume, the glucose solution was administered over a period of 2 minutes.

Jugular blood samples were collected before and at intervals after the administration of glucose. These samples were retained for glucose and VFA analyses. The chemical methods used were those described in Chapter I. Control trials using sterile 0.9 % NaCl (1 ml/kg of body weight) were conducted in the case of calves 7 and 8.

## Results

Changes in the glucose and volatile fatty acid concentrations of jugular blood following the intravenous injection of 50 % (w/v) D-glucose and following the intravenous injection of 0.9 % NaCl (table 20, fig. 14 and 15). The intravenous administration of D-glucose was followed by an increase in the concentrations of both glucose and VFAs in the jugular blood (table 20, fig. 14). Greater increases in jugular glucose and VFA levels were observed in calves 5 and 6, which received 1 gm of D-glucose/kg of body weight, than in calves 7 and 8, which were given 0.5 gm of D-glucose/kg of body weight (table 20, fig. 14). The severity of the hyperglycaemia did not however appear to be proportional to the dose rate of glucose administered. In each of the four calves tested the elevated jugular glucose and VFA levels returned to the fasting concentrations at similar rates (table 20, fig. 14).

Only minor fluctuations in the glucose and VFA concentrations of jugular blood were observed in control experiments in which 0.9 % NaCl was administered instead of the 50 % (w/v) D-glucose solution.

Experiment III. A Hyperglycaemia Induced by the Intravenous  
Injection of Adrenaline

Materials and Methods

Changes in the glucose and VFA concentrations of jugular blood following the intravenous administration of adrenaline were studied in calves 7, 8, 9 and 10. Details of the breed, age, sex and weight are shown in table 21.

TABLE 21

Details concerning the breed, age, sex and weight of the calves used in the adrenaline experiment.

Galf	Breed	Age days	Sex	Weight kg
7	Jersey	107	Male	68
8	Jersey	106	Male	60
9	Jersey	122	Male	65
10	Jersey	115	Male	63

All four calves had been previously weaned onto pasture, a mixture of white clover, short-rotation and long-rotation rye grass. Jugular vein catheters were introduced, as was described in Chapter I, on the day preceding the experiments. The calves were fasted for a period of 24 hours prior to the administration of adrenaline. A dose rate of 5  $\mu$ gm of adrenaline (May and Baker)/kg of body weight was given to all the calves. The adrenaline was first made up to a volume of 10 ml with sterile 0.9 % NaCl, and was then injected through the jugular vein catheter over a period of 1 minute. Samples of jugular blood were collected before and at 2, 10 and 20 minutes after the administration of adrenaline. These blood samples were later analysed for their glucose and VFA concentrations by the methods described

TABLE 22

Changes in the glucose and volatile fatty acid concentrations of jugular blood following the intravenous injection of adrenaline.

C A L F	JUGULAR BLOOD GLUCOSE mg/100 ml				JUGULAR BLOOD VFA m-moles/lit			
	0 min	2 min	10 min	20 min	0 min	2 min	10 min	20 min
7	39.94	42.53	47.70	35.91	2.17	2.20	2.15	2.51
8	30.75	48.27	43.39	40.80	1.90	1.98	2.62	2.13
9	53.57	66.29	64.51	61.16	2.06	2.67	2.39	2.41
10	43.08	44.87	51.12	55.13	1.94	2.02	1.94	2.26

TABLE 23

Changes in the glucose and volatile fatty acid concentrations of jugular blood following the intravenous injection of 0.9 % NaCl.

C A L F	JUGULAR BLOOD GLUCOSE mg/100 ml				JUGULAR BLOOD VFA m-moles/lit			
	0 min	2 min	10 min	20 min	0 min	2 min	10 min	20 min
7	41.87	41.57	41.57	43.37	1.94	2.11	1.76	2.18
8	29.52	28.31	30.42	32.22	1.34	1.63	1.66	1.96
9	47.74	50.00	50.00	48.49	1.81	1.85	1.93	1.91
10	44.22	43.22	45.98	44.72	2.29	2.11	2.30	2.09

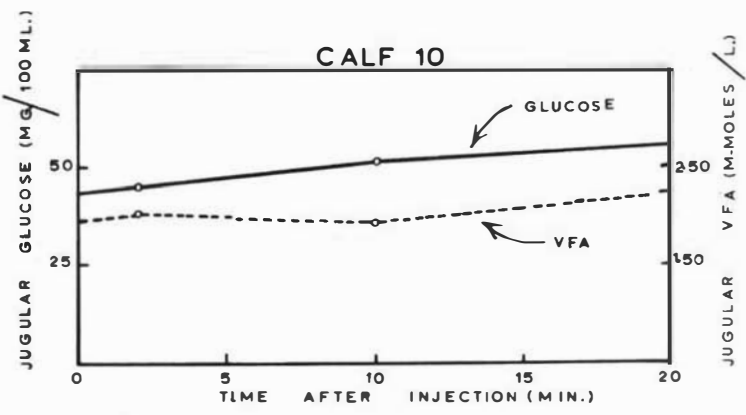
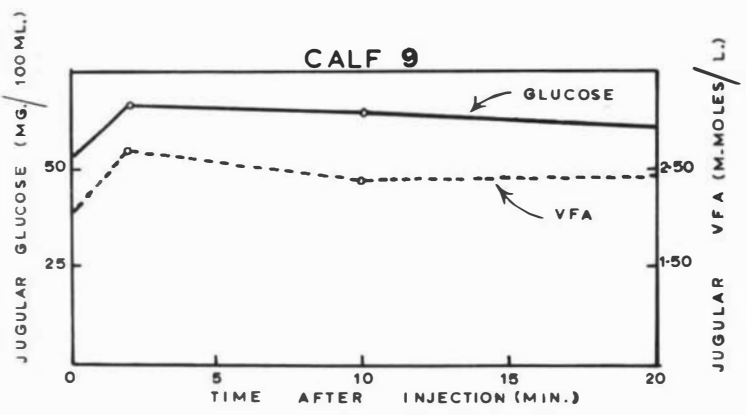
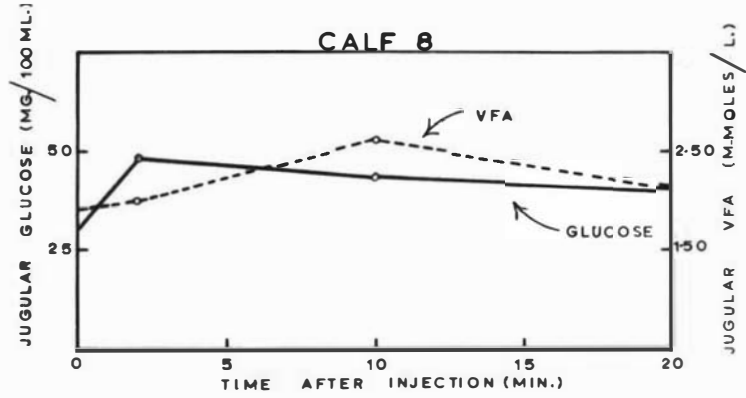
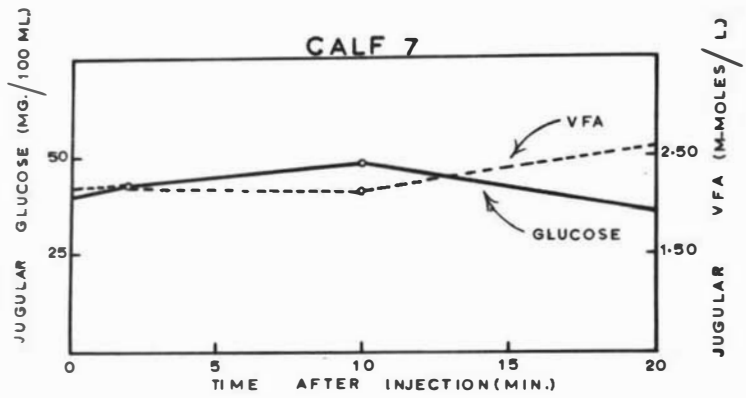


Fig. 16. Changes in the glucose and volatile fatty acid concentrations of jugular blood following the intravenous injection of adrenaline.



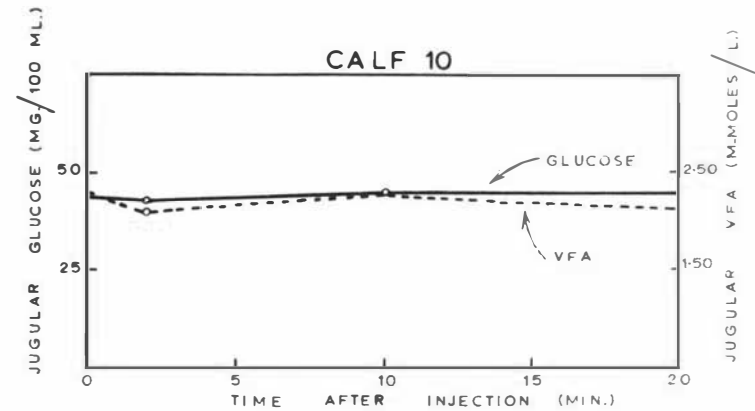
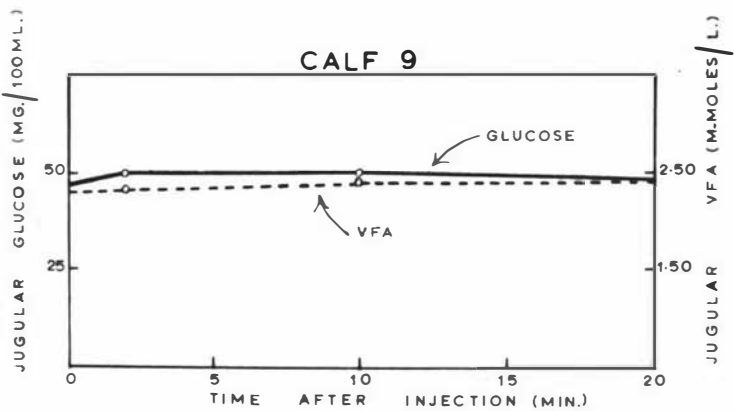
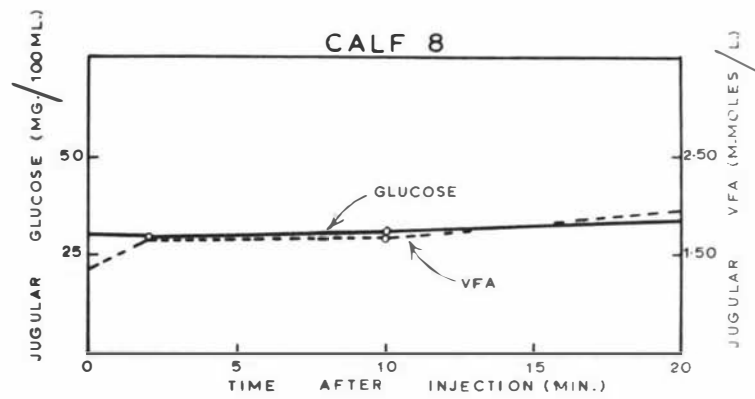
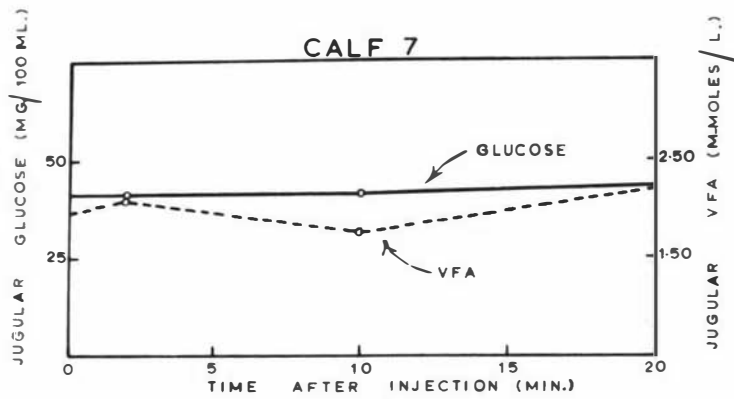


Fig. 17. Changes in the glucose and volatile fatty acid concentrations of jugular blood following the intravenous injection of 0.9% NaCl.



in Chapter I.

Control experiments were performed in all the calves one week after the adrenaline trials. The procedures used in these control experiments were similar to those described above, except that 10 ml of 0.9 % NaCl containing no adrenaline was administered.

## Results

Changes in the glucose and volatile fatty acid concentrations of jugular blood following the intravenous injection of adrenaline and following the intravenous injection of 0.9 % NaCl (tables 22 and 23, fig. 16 and 17).

The levels of jugular blood glucose in the present experiments were apparently not affected by either the injection of solutions into or the collection of blood samples from the jugular vein. Only minor fluctuations in the glucose concentrations of jugular blood were noted in the control experiments when 0.9 % NaCl was administered (table 23, fig. 17). In contrast definite increases in the glucose concentrations of jugular blood were recorded in all the calves following the administration of adrenaline (table 22, fig. 16). These induced hyperglycaemias followed different time-courses in the individual calves. In calves 8 and 9 the highest glucose concentration was detected 2 minutes following the administration of adrenaline and after 10 minutes and 20 minutes in calves 7 and 10 respectively (table 22, fig. 16).

Increases in the VFA concentration of jugular blood following the intravenous administration of adrenaline were demonstrated in all four calves (table 22, fig. 16). These increases, although apparently more delayed, nevertheless seemed to parallel the rise in blood glucose concentrations.

## DISCUSSION

### Experiment I. A Hyperglycaemia Induced by the Feeding of Skim Milk Supplemented with Lactose (tables 17 and 18, fig. 10, 11, 12 and 13).

The VFA concentrations and pH changes of the rumen observed following the feeding of 4 lit of either whole milk or skim milk supplemented with lactose (table 17, fig. 10 and 11) indicate the entry of a portion of these test diets into the rumen. Both Warner et al. (1956) and Tamate et al. (1962) have demonstrated that the abomasal capacity in calves of a similar age and breed to calf 2 is considerably less than 4 lit. Comline and Titchen (1951) have shown that abomasal distention in decerebrate preparations leads to an inhibition of the reflex closure of the oesophageal groove with the consequent entry of fluids into the rumen.

Bryant et al. (1958) demonstrated the persistence of lactic acid-producing bacteria in the rumen of milk-fed calves with advancing age. Consumption of large quantities of soluble carbohydrate by mature ruminants is frequently followed by a retarded rate of VFA production and by a rapid and precipitous decline in rumen pH. This atypical fermentation is attributed to the rapid proliferation of lactic acid-producing micro-organisms (Annison and Lewis, 1959). In the present study only minor increases in the VFA levels of the rumen were observed following the feeding of both test diets (table 17, fig. 10 and 11). The pH changes of the rumen, however, did not appear to be solely dependent on its VFA concentrations, since a greater decline in pH was recorded with skim milk supplemented with lactose than with whole milk (table 17, fig. 10 and 11). These observations suggest a predominantly lactic acid-producing fermentation in the rumen of the 6-week-old milk-fed calf used in the present study, and are in agreement

with the findings of Bryant et al. (1958).

Post-prandial increases in portal blood VFA levels were observed with both test diets (table 18, fig. 12 and 13). It is unlikely that these increases were wholly caused by VFAs originating in the rumen, since only minor elevations in rumen VFA concentrations were recorded. Hungate et al. (1959) have demonstrated that the caecum contributes less than 5 % of the total VFAs produced in the alimentary tract of mature ruminants. On the basis of this observation, it is also improbable that the caecum could have, by itself, accounted for all the portal blood VFA concentration changes observed in the present study. The absence of a substantial transfer of VFAs from the alimentary tract therefore suggests that the enhanced post-prandial VFA concentrations of portal blood noted after the feeding of both test diets originated largely from endogenous sources.

Greater post-prandial increases in both the glucose and VFA concentrations of portal blood were recorded after the feeding of skim milk supplemented with lactose than when whole milk was fed (table 18, fig. 12 and 13). These observations indicate that the post-prandial increases in portal blood VFA levels were at least in part caused by the metabolism of absorbed dietary glucose.

The complications associated with the chronic catheterization of the portal vein (Chapter I) precluded the repetition of this experiment in other calves.

Experiment II. A Hyperglycaemia Induced by the Intravenous Injection of Glucose (table 20, fig. 14 and 15).

Both McCandless and Dye (1950) and Warner et al. (1959) conducted glucose tolerance tests in calves. Neither group of workers, however, studied the possible relationship between the levels of circulating glucose and VFAs in peripheral blood. Evidence of such a relationship is presented in the present study by:-

1. The simultaneous increases in both glucose and VFA concentrations of jugular blood observed in all four calves following the intravenous administration of glucose into the jugular vein (table 20, fig. 14).
2. The gradual and similar return of both these enhanced concentrations to their pre-injection levels in all four calves (table 20, fig.14).

Experiment III. A Hyperglycaemia Induced by the Intravenous Injection of Adrenaline (table 22, fig. 16).

Further evidence to indicate a relationship between the levels of circulating glucose and VFAs in jugular blood is presented in this study by the intravenous administration of adrenaline. Although the time-course of the adrenaline-induced hyperglycaemias differed in the individual calves, they were nevertheless accompanied by increased, but frequently delayed, concentrations of VFAs (table 22, fig. 16). This observation is in agreement with the findings of Lindsay (1961) who observed an increase in the concentrations of plasma acetate following the intravenous infusion of adrenaline in sheep.

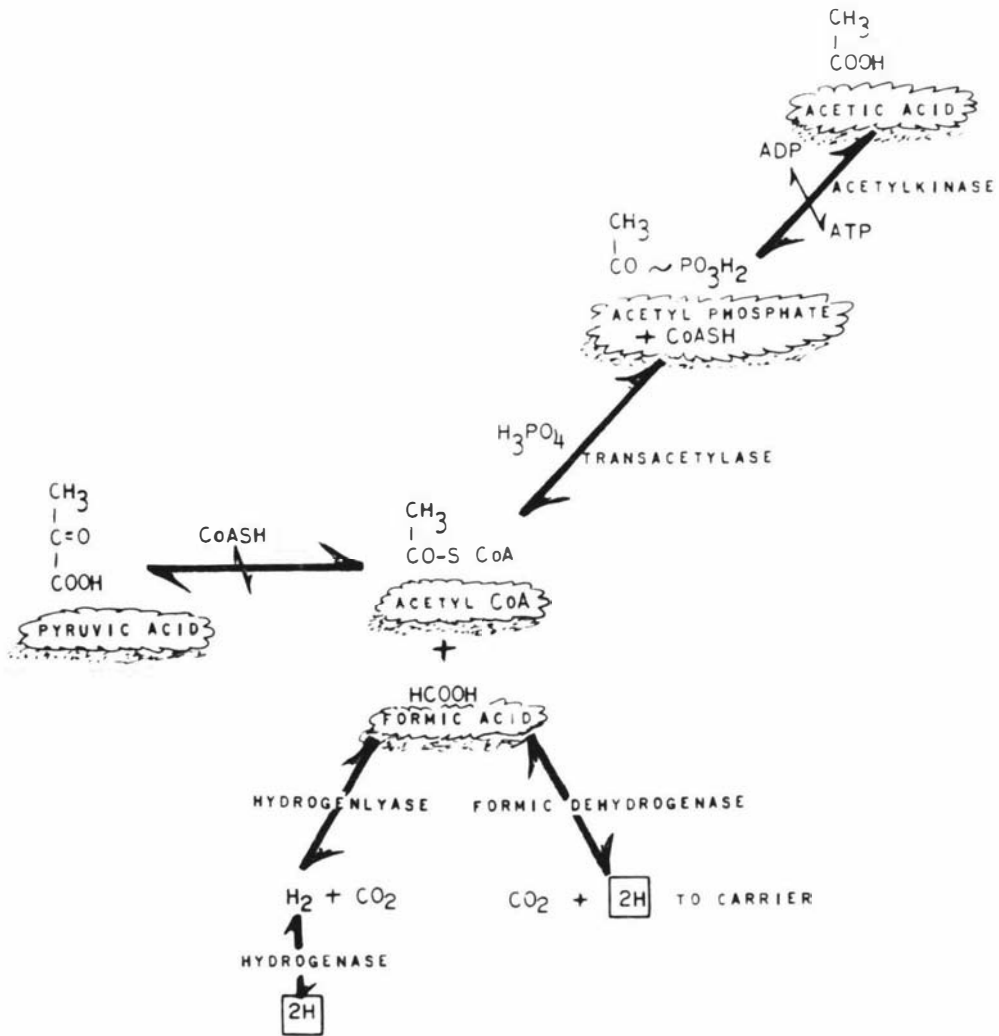


Fig. 18. The formation of acetic acid from acetyl coenzyme A. (Unbreit, Metabolic Maps, Vol. II, Burgess).

## General

In the present study the VFA concentrations of blood were determined by steam distillation of the concentrated protein-free filtrates of blood and the titration of the distillates with standard alkali. No attempt was made to identify the components of the distillates. It is unlikely that the distillates contained high levels of interfering compounds, e.g. lactate, pyruvate, B-hydroxybutyrate and chloride since the analytical procedure (Annison, 1954) was specifically designed to both minimize their effects and prevent the breakdown of formate. The VFA concentration changes observed in all three experiments were therefore probably a reflection of changes in the circulating levels of formic, acetic and propionic acids.

In vitro studies indicate that the metabolic pathways of carbohydrates, lipids and proteins converge and meet on common ground in acetyl coenzyme A (acetyl CoA), the biologically active form of acetate, and that any interference with the metabolism of any one of these three nutrients is likely to be mediated to the other two through acetyl CoA. It is also reasonable to expect a state of equilibrium to exist between intracellular acetyl CoA and extracellular free acetate. An induced hyperglycaemia could conceivably lead to a saturation of glucose metabolic pathways. This saturation could in turn result, through a possible temporary shortage of either CoA or oxaloacetate, in increased levels of free acetate and formate in the blood (fig. 18).

The importance of glucose in the energy metabolism of simple-stomached animals is well-established. Circulating glucose in these animals has been shown to originate in both the alimentary tract and in the glycogen reserves of the body, and its level has been demonstrated to be under the control of

a number of hormones that include insulin, glucagon, adrenaline, growth hormones and adrenal steroids. The glucose concentration of the blood at any one time represents the end-result of a complex of at least four identifiable physiological processes, namely, intestinal absorption of glucose, glucose mobilization from glycogen reserves, glucose uptake by the tissues and the neuro-endocrine control of these three processes.

Although glucose absorption from the alimentary tract of mature ruminants is limited (Schanbye, 1951c; Annison et al., 1957; Fries and Conner, 1960; Bensadoun et al., 1962) and despite the fact that these animals have comparatively low levels of circulating glucose in their blood (McCandless and Dye, 1950), glucose nevertheless plays as important a role in their energy metabolism as it does in simple-stomached animals. Recent radio-isotope infusion studies indicate that the glucose utilization rates per unit of metabolic body-size are of the same magnitude in mature ruminants as those found in monogastric animals (Annison and White, 1961; Davis and Brown, 1962; Ford, 1964). In the absence of a substantial supply of glucose from the alimentary tract, Lindsay (1959 b) suggests that the greater part of circulating glucose found in the blood of mature ruminants is formed by gluconeogenesis from absorbed dietary propionate.

The levels of circulating VFAs have, until recently, been considered to be largely dependent on the concentrations of these acids in the rumen (Masson and Phillipson, 1951; Tsuda, 1956; Annison et al., 1957; Dobson, 1961; Annison, 1965), and to be independent of endocrine control (Lindsay, 1961). Evidence has however accumulated to suggest that the situation is more complex. Substantial endogenous contributions of VFAs to the circulation were revealed by the radio-isotope studies of Annison and White (1962), and Lee and Williams (1962). The experiments of Jarrett

and Potter (1957), Reid (1958), Lindsay (1959a), Jarrett and Filsell (1961) and Lindsay (1961), as well as those described in this chapter, suggest that the circulating levels of these endogenous VFAs are regulated, at least in part, by the glucose concentrations of the blood, and may therefore be under indirect endocrine control.

Attempts to investigate the effects of advancing age and diet on the absorption of VFAs from the rumen of conscious calves by the analysis of portal blood were complicated by the difficulties encountered in maintaining the patency of portal vein catheters and by the endogenous contributions of VFAs to the circulation. These complications did not, however, exclude the possibility that VFA transport across the rumen wall could still be studied, in conscious calves, either by the appearance of these acids in the peripheral circulation, or by their disappearance from the rumen; and in anaesthetized preparations, by the analysis of rumen effluent blood.

#### SUMMARY

1. Three experiments were performed to determine the effects of an induced hyperglycaemia on the concentration of circulating VFAs in calves.
2. The level of glucose in the blood was enhanced by:-
  - (a) the feeding of skim milk supplemented with lactose (Experiment I)
  - (b) the intravenous administration of glucose (Experiment II)
  - (c) the intravenous administration of adrenaline (Experiment III)
3. These experiments demonstrated:-
  - (a) the presence of endogenous sources of circulating VFAs
  - (b) a relationship between the levels of circulating glucose and VFAs.

4. The experiments were discussed in the light of the literature.
5. The experiments suggested:-
  - (a) the investigation of VFA absorption in conscious calves by the analysis of peripheral blood.
  - (b) the introduction of preformed VFAs into the rumen in future studies.

## CHAPTER III

### STUDIES ON THE ABSORPTION AND METABOLISM OF PREFORMED VOLATILE FATTY ACIDS IN CONSCIOUS CALVES BY THE ANALYSIS OF JUGULAR BLOOD

The investigation of VFA absorption from the rumen by analysis of portal blood proved to be impractical in conscious calves (Chapter I). Long-term comparative studies in the same calves were precluded by the difficulties encountered in maintaining patent portal vein catheters. Experiments on four separate calves, of different ages and in various stages of rumen development, demonstrated post-prandial increases in portal blood VFA levels following whole-milk feeding (Chapter I). These experiments suggested the introduction of preformed salts of the VFAs into the rumen in future experiments concerned with the absorption of these acids from the fore-stomach. A relationship between the circulating concentrations of glucose and VFAs in the blood was also noted in these experiments (Chapters I and II) and warranted further investigation in calves.

Sutton et al. (1963) were able to demonstrate acetic acid absorption from the rumen by changes in the levels of this acid relative to polyethylene glycol (a marker) in a solution containing known concentrations of these compounds following its introduction into the rumen (page 10).

They could not, however, detect the appearance of acetic acid in jugular blood. Sutton and his colleagues introduced test solutions into the starved and emptied rumen through a rumen fistula, and prevented the entry of saliva into the fore-stomach by inflating a balloon in the oesophagus. These procedures have all been shown to decrease VFA transport, and the curtailment of these procedures could conceivably result in a greater rate of absorption than was noted in the studies of Sutton and his co-workers.

The metabolism of VFAs by rumen epithelium was studied by Pennington (1952, 1954) who also showed that the epithelium has a greater preference for butyrate and propionate than for acetate. Introduction of butyric and propionic acids together with acetic acid into the rumen could possibly enhance the metabolic activity of rumen epithelium (Armstrong, Blaxter and Graham, 1957) and result in an increased rate of VFA transport.

Although the studies of Schambye (1951 a) and Annison et al. (1957) demonstrated rapid hepatic removal of absorbed VFAs from the peripheral circulation in adult ruminants, the experiments of Sutton et al. (1963) suggested that it might be feasible to study VFA absorption from the rumen by the analysis of peripheral blood if the following modifications to their technique were adopted, namely:-

- (a) a buffered VFA solution to be introduced into the rumen following a 24-hour period of fasting off milk and 48 hours off pasture, instead of into the partially isolated, washed and emptied rumen of starved animals;
- (b) the introduction into the rumen of a buffered solution containing the salts of acetic, propionic and butyric acids instead of acetic acid only.

The effects of advancing age and diet on VFA absorption and metabolism were investigated, in the experiments described in this chapter, by the analysis of jugular blood. Two pairs of Friesian bull-calves were used in these experiments. One pair was reared on a diet of milk only (Milk Fed Calves No. 11 and 12) and the other pair was fed milk and had continuous access to pasture from the age of 10 days onwards (Pasture Fed Calves No. 13 and 14). Absorption was studied by the appearance of VFAs in jugular blood following the introduction into the rumen of a buffered solution containing the salts of acetic, propionic and butyric acid (Experiment IV). Utilization was investigated by the clearance of intravenously imposed loads of VFAs from the peripheral circulation (Experiment V).

#### REVIEW OF LITERATURE

##### Absorption of Preformed Volatile Fatty Acids from the Rumen of Conscious Calves Studied by the Analysis of Jugular Blood

Martin et al. (1959) and Sutton et al. (1963) studied the absorption of VFAs from the rumen of conscious calves by the analysis of jugular blood. The results of their studies were reviewed on pages 7-10.

##### Metabolism of Preformed Volatile Fatty Acids in Conscious Calves Studied by the Analysis of Jugular Blood

Volatile fatty acid metabolism was originally investigated in adult ruminants by means of intravenous tolerance tests. A serious limitation to this experimental technique is that VFA utilization can only be studied under unphysiologically high and continuously declining circulating

concentrations (Annison and Lindsay, 1961).

Jarrett, Potter and Filsell (1952) and Pugh and Scarisbrick (1952) were the first to report on acetate tolerance tests in ruminants. Both groups of workers demonstrated that the clearance of intravenously imposed loads of acetate from the peripheral circulation was slower in mature sheep than in either cats (Smyth, 1947) or dogs (Ciaranfi and Fornesu, 1954). Jarrett and his co-workers injected acetate over a period of 5-10 minutes at a dose of 5 m-moles/kg of body weight and showed that blood acetate returned to within 10 mg % of the pre-injection level within 70 minutes. Pugh and Scarisbrick injected a solution of sodium acetate into the peripheral circulation of normal and ketotic sheep at a dose of 8.3 m-moles/kg of body weight and observed similar acetate tolerance rates in the control sheep to those reported by Jarrett et al. (1952). Acetate utilization was depressed in the sheep suffering from ketosis.

Jarrett and Potter (1957) undertook acetate, propionate and butyrate tolerance tests on normal sheep and on sheep made diabetic by the injection of alloxan and by pancreatectomy. These workers observed a delay in the clearance of injected VFAs from the systemic circulation of the diabetic sheep. The delay was more pronounced with acetate than with either propionate or butyrate. Partial failure of diabetic sheep to utilize VFAs was suggested as a likely explanation for these observations.

Acetate tolerance tests on sheep fed different diets were performed by Reid (1958), who noted that the disappearance of injected acetate from the peripheral circulation was affected by the nature of the diet and by fasting. The modifying effects of the diet on acetate tolerance in sheep was later confirmed by the experiments of Jarrett and Filsell (1960).

Jarrett and Filsell (1961) conducted acetate tolerance tests in normal sheep and in sheep made hyperglycaemic by the intravenous injection of glucose. They noted that acetate clearance was more rapid in the hyperglycaemic sheep than in the control animals, and suggested that the modifying effect of the diet on acetate tolerance noted earlier by Reid (1958) and Jarrett and Filsell (1960) might have been caused by an enhanced ruminal production of gluconeogenic propionate from low-fiber feeds.

The effect of dietary protein supplements on the tolerance of sheep to acetate and propionate was studied by Egan (1965). An improvement in the utilization of intravenously administered acetate was observed in sheep fed ad libitum low-quality oaten chaff when the diet was supplemented with casein or when casein was infused directly into the duodenum. Propionate tolerance was also improved by the addition of casein to the low-protein oaten chaff diet.

Circulating VFAs in the blood of mature ruminants were originally considered to arise largely from exogenous sources located mainly in the rumen and to a lesser extent in the caecum and colon also. Recent radio-isotope studies (Davis et al., 1960; Annison and White, 1962; Lee and Williams, 1962) have however indicated the endogenous origin of a substantial portion (25-40 %) of these circulating VFAs. The experiments of Annison and Lindsay (1961), Sabine and Conner Johnson (1964) and Conner Johnson et al. (1965) have further shown that the turnover rates of pool acetate in adult ruminants are extremely rapid, ranging from 2-4 minutes.

No reference was found in the literature consulted to any studies on the tolerance of young calves to intravenously imposed loads of the VFAs. Young, Tove and Ramsey (1964) used radio-isotope techniques to investigate

the effect of advancing age on VFA metabolism in calves fed on milk only. These workers infused 1-C<sup>14</sup> acetate, -propionate and -butyrate into the circulation of milk-fed calves and monitored the expired air for C<sup>14</sup>O<sub>2</sub> activity. Although they did not mention the lower age of the animals, their results indicated that the milk-fed calves up to the age of 80 days were capable of metabolizing large quantities of VFAs. Young and his colleagues were unable to detect any significant differences in the metabolism of these acids that could be attributed to either an individual acid or to the age of the calves.

## EXPERIMENTAL

### Experimental Animals

Four Friesian bull-calves were randomly divided into two pairs following a 2-day period of colostrum feeding. One pair of calves (No. 11 and 12) was reared on a diet of milk only (Milk Fed Calves) and the other pair (No. 13 and 14) had, in addition to milk, continuous access to pasture from the age of 10 days onwards (Pasture Fed Calves). All the calves were bucket-fed whole Jersey milk twice daily at the rate of 12.5 % of body weight. A teaspoon of mineralized salt, the composition of which is presented in table 24, was dissolved in the milk of each calf at the morning feeding.

TABLE 24

Trace mineralized salt mixture, Feed Trade Manual Edition No. 6, 1957, published by National Miller Publication Inc., 6 E. McDonald Rd., Prospect Heights, Ill.

Ingredient	%
Iodized Salt	99.0
Cobalt Carbonate	0.062
Copper Sulfate	0.25
Ferrous Sulfate	0.188
Ferrio Citrate	0.187
Manganese Sulfate	0.313
<u>Calculated Analysis</u>	
Cobalt	0.013
Copper	0.020
Iron	0.05
Manganese	0.10
NaCl	99.0

Milk-fed calves were reared in concrete pens on slatted wooden frames. Pasture-fed calves were weaned at the age of 70 days after reducing the daily milk allowance during the eighth and ninth week to 6 % of body weight. The calves were weighed at weekly intervals, and their milk intake adjusted accordingly (table 25).

A Jarrett cannula was introduced into the rumen when the calves were 6-8 days old. The packed cell volume and haemoglobin concentration (Sahli's Method) of blood were determined at regular intervals with advancing age (table 25).

TABLE 25

Details concerning the growth rates of the calves together with the haemoglobin concentrations and the packed cell volume of their blood.

G R O U P	Galf No.	Growth Rate Kg/Day			Haemoglobin Gm/100 ML Blood				Packed Cell Volume %			
		Birth to 40 days	40-75 days	75-110 days	5 days	30 days	65 days	100 days	5 days	30 days	65 days	100 days
M I L K	11	0.52	0.79	0.87	10.5	10.5	7.0	8.0	35	33	26	28
	12	0.64	0.92	0.58	14.5	13.0	9.0	9.5	51	46	31	36
P A S T U R E	13	0.69	0.88	0.36	11.0	12.0	11.5	10.5	37	39	40	36
	14	0.65	0.82	0.26	15.0	13.0	11.5	11.5	54	42	41	40

### Fistulation of the Rumen

The rumen was fistulated under general anaesthesia following a 24-hour period of fasting. Atropine sulphate was subcutaneously administered at a dose of 1 mg/10 kg of body weight. Anaesthesia was induced with Halothane (Fluothane, Imperial Chemical Industries) by the open mask technique and maintained with ether after endotracheal intubation.

Entry into the abdominal cavity was gained through a 5 cm paracostal skin incision located on the dorsal aspect of the left paralumbar fossa. Allis forceps were used to align the posterior dorsal sac of the rumen with the skin incision. The rumen wall was sewn, along both sides of the skin incision, to peritoneum and abdominal muscle layers, with a continuous catgut suture. A vertical incision, approximately 4 cm long, was then made in the exposed rumen wall, the edges of which were in turn sewn to the skin with interrupted nylon monofilament thread sutures.

A Jarrett (1948) cannula was introduced into the fistulated rumen and retained in position with a polyvinylchloride washer. Prophylactic medication against infection was started upon the completion of surgery and continued for 4-6 days post-operatively. This entailed the daily intra-muscular injection of one million units of penicillin (Benethamine Penicillin, Glaxo) and the dusting of surgical wounds with neomyoin and bacitracin powder (Cicatrin, Calmio).

### Catheterization of the Jugular Vein

Jugular vein catheters were introduced as was described in Chapter I.

Experiment IV. Absorption of Preformed Volatile Fatty Acids from the Rumen of Conscious Calves Studied by the Analysis of Jugular Blood

Volatile fatty acid absorption tests were conducted at the age of 40, 75 and 110 days in the two pairs of Friesian bull-calves described earlier. The object of the experiment was to determine the effects of advancing age and diet on the absorption of VFAs from the rumen by changes in the VFA concentration of jugular blood following the introduction of a buffered solution of these acids into the rumen.

The tests were performed at the end of a 24-hour period of fasting off milk and 48 hours off pasture. Jugular blood samples were collected before and 30, 60 and 120 minutes after the buffered solution of VFAs was introduced into the rumen. Samples of rumen contents were also taken before, and 10 and 120 minutes after the VFAs were introduced. Blood and rumen samples were analysed for VFAs by the methods described in Chapter I.

A 0.5 M solution containing 75 % acetic, 20 % propionic and 5 % butyric acids buffered to pH 6.00 in Krebs Ringer (where the VFAs replaced the NaCl) and Sorensen's phosphate buffer was used (table 26). The molar proportions of the VFAs were similar to those found in the adult rumen at the peak of fermentation (Annison et al., 1957). This solution was administered at a dose of 20 ml/kg of body weight (10 m-moles/kg of body weight) and was introduced into the rumen through the Jarrett cannula. No attempt was made to either empty and wash the rumen or to isolate it from the proximal and distal portions of the alimentary tract. The pH of the rumen was maintained within the range of 6.00-6.20 by the continuous infusion of 5 N phosphoric acid into the rumen. Rumen pH was determined at 10-minute intervals in order to be able to regulate the phosphoric acid

drip. These procedures were simplified by passing three polyvinylchloride tubes into the rumen through separate holes in the rubber stopper of the Jarrett cannula. One tube was attached to a glass funnel and served to introduce the VFA solution into the rumen. The second tube was attached to a plastic wash-bottle and was used to aspirate rumen contents. The third tube was attached to a blood transfusion apparatus for the continuous infusion of phosphoric acid.

TABLE 26

The chemical composition of 5 lit of the volatile fatty acid solution introduced into the rumen.

Ingredients	Proportion per cent	Molarity	Quantity gm
<u>Volatile Fatty Acids</u>	-	0.500	-
Acetic acid	70	-	105.1
Propionic acid	25	-	46.3
Butyric acid	5	-	11.0
<u>Krebs Ringer</u>			
Potassium chloride (KCl)	-	0.005	1.8638
Calcium chloride (CaCl <sub>2</sub> .6H <sub>2</sub> O)	-	0.003	3.2864
Magnesium sulphate (MgSO <sub>4</sub> .7H <sub>2</sub> O)	-	0.001	1.2325
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	-	0.001	0.6805
<u>Sorensen's Phosphate Buffer</u>	-	0.01	-
Disodium hydrogen phosphate (Na <sub>2</sub> HPO <sub>4</sub> .2H <sub>2</sub> O)	-	-	1.0688
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	-	-	0.5991

The 0.5 M solution of VFAs (Sutton et al., 1962) was prepared in the following manner:- The fatty acids (B.D.H., Analar) were diluted in 1 lit of distilled water and adjusted to a pH of 6.00 with 4 N NaOH. Krebs Ringer and Sorensen's buffer ingredients were dissolved in another 1 lit of distilled water and added to the fatty acid solution. The volume was

made up to 5 lit with distilled water and the pH again adjusted to 6.00 by the addition of 1-2 ml of 4 N NaOH. This solution was stored at a temperature of 4°C and was warmed to a temperature of 37°C prior to its introduction into the rumen.

Experiment V. Metabolism of Preformed Volatile Fatty Acids in Conscious Calves Studied by the Analysis of Jugular Blood

Intravenous VFA tolerance tests were conducted at the age of 35, 70 and 105 days in the two pairs of Friesian bull-calves described earlier. The object of these tests was to determine the effects of advancing age and diet on the utilization of VFAs in calves.

The tests were performed at the end of a 24-hour period of fasting off milk and 48 hours off pasture. Jugular blood samples were collected for VFA analysis before and 5, 30, 45, 60, 90 and 120 minutes after the intravenous injection of a 4 M solution of VFAs into the jugular vein. In order to simulate the post-prandial proportions of VFAs in portal blood the VFA solution contained 85 % acetic and 15 % propionic acid (Armison et al., 1957). The VFAs were administered at a dose of 4 m-moles/kg of body weight over a 2-minute period irrespective of volume.

One lit of the VFA solution (table 27) was prepared in the following manner:- The acids (B.D.H., Analar) were weighed out and diluted in 400 ml of distilled water. This solution was titrated to a pH of 7.40 with 10 N NaOH and made up to just under 1 lit with distilled water. The pH was again adjusted to 7.40 by the addition of a few drops of 10 N NaOH, and the volume made up to 1 lit with distilled water. The solution was autoclaved at 115°C for 30 minutes and stored at a temperature of 4°C.

TABLE 27

The composition of 1 lit of the volatile fatty acid solution used in the volatile fatty acid tolerance tests.

Volatile Fatty Acid	Proportion per cent	Quantity gm
Acetic acid	85	204.2
Propionic acid	15	44.5

RESULTS

Fasting Levels of Volatile Fatty Acids in Jugular Blood (table 28)

The fasting levels (24 hours off milk and 48 hours off pasture) of VFAs in jugular blood ranged from 0.83-2.32 m-moles/lit (table 28). These levels varied in calves of the same age and on the same diet, and were also different in any one individual animal following identical fasting periods which were only 5 days apart (table 28). There was no apparent fasting VFA concentration of jugular blood characteristic of either the age or the diet of the calves.

TABLE 28

The volatile fatty acid concentration of jugular blood following 24 hours of fasting off milk and 48 hours off pasture.

Group	Calf No.	Age days	Jugular VFAs m-moles/lit	Age days	Jugular VFAs m-moles/lit
MILK	11	35	1.15	40	0.95
		70	1.96	75	1.44
		105	1.55	110	1.53
	12	35	0.83	40	1.19
		70	1.93	75	2.32
		105	0.91	110	1.02
PASTURE	13	35	0.94	40	0.90
		70	0.83	75	1.21
		105	1.56	110	1.33
	14	35	1.05	40	1.11
		70	0.95	75	1.01
		105	1.58	110	1.67

TABLE 29

Changes in the volatile fatty acid concentrations of jugular blood and rumen liquor following the introduction of a buffered test solution of volatile fatty acids into the rumen, together with the amount of 5 N phosphoric acid required to maintain the pH of the rumen between 6.00 and 6.20.

Group	Calf No.	Age days	Weight kg	Infusate ml	JUGULAR VFAs m-moles/lit				RUMEN LIQUOR VFAs m-moles/lit			5 N H <sub>3</sub> PO <sub>4</sub> ml
					0 min	30 min	60 min	120 min	0 min	10 min	120 min	
M I	11	40	58	1160	0.95	0.91	1.18	1.18	11.12	222.72	154.99	40
		75	84	1680	1.44	1.58	1.50	1.50	23.38	209.83	161.53	65
		110	116	2320	1.53	1.50	1.42	1.44	10.22	381.40	174.70	65
L K	12	40	61	1220	1.19	1.56	1.46	0.89	10.38	245.55	149.05	50
		75	90	1800	2.32	2.52	1.84	2.40	8.84	448.71	224.03	70
		110	107	2140	1.02	1.14	1.26	1.40	8.70	390.20	223.29	50
P A S T U R E	13	40	65	1300	0.90	-	1.08	1.04	12.65	276.27	124.12	85
		75	90	1800	1.21	1.39	1.27	1.29	12.68	213.31	109.58	200
		110	110	2200	1.33	1.73	1.79	1.62	10.02	115.30	73.17	395
	14	40	62	1240	1.11	1.14	1.20	1.04	21.08	231.57	114.98	75
		75	86	1720	1.01	1.39	1.49	1.71	10.98	122.48	83.57	235
		110	90	1800	1.67	1.84	2.33	1.95	14.65	114.76	76.08	335

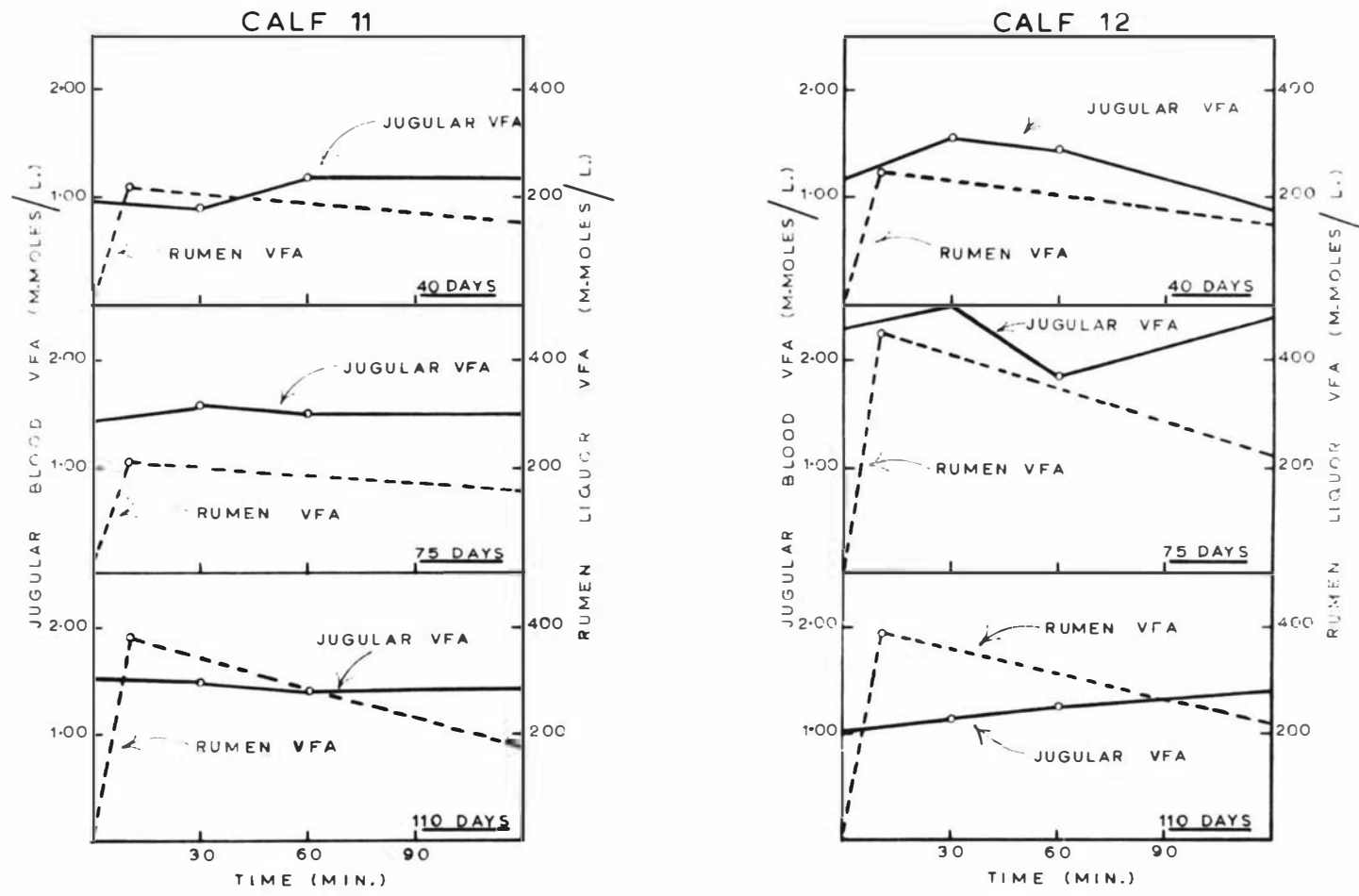


Fig. 19. Changes in the volatile fatty acid concentrations of rumen contents and jugular blood following the introduction of preformed volatile fatty acids into the rumen of the Milk Fed Calves.

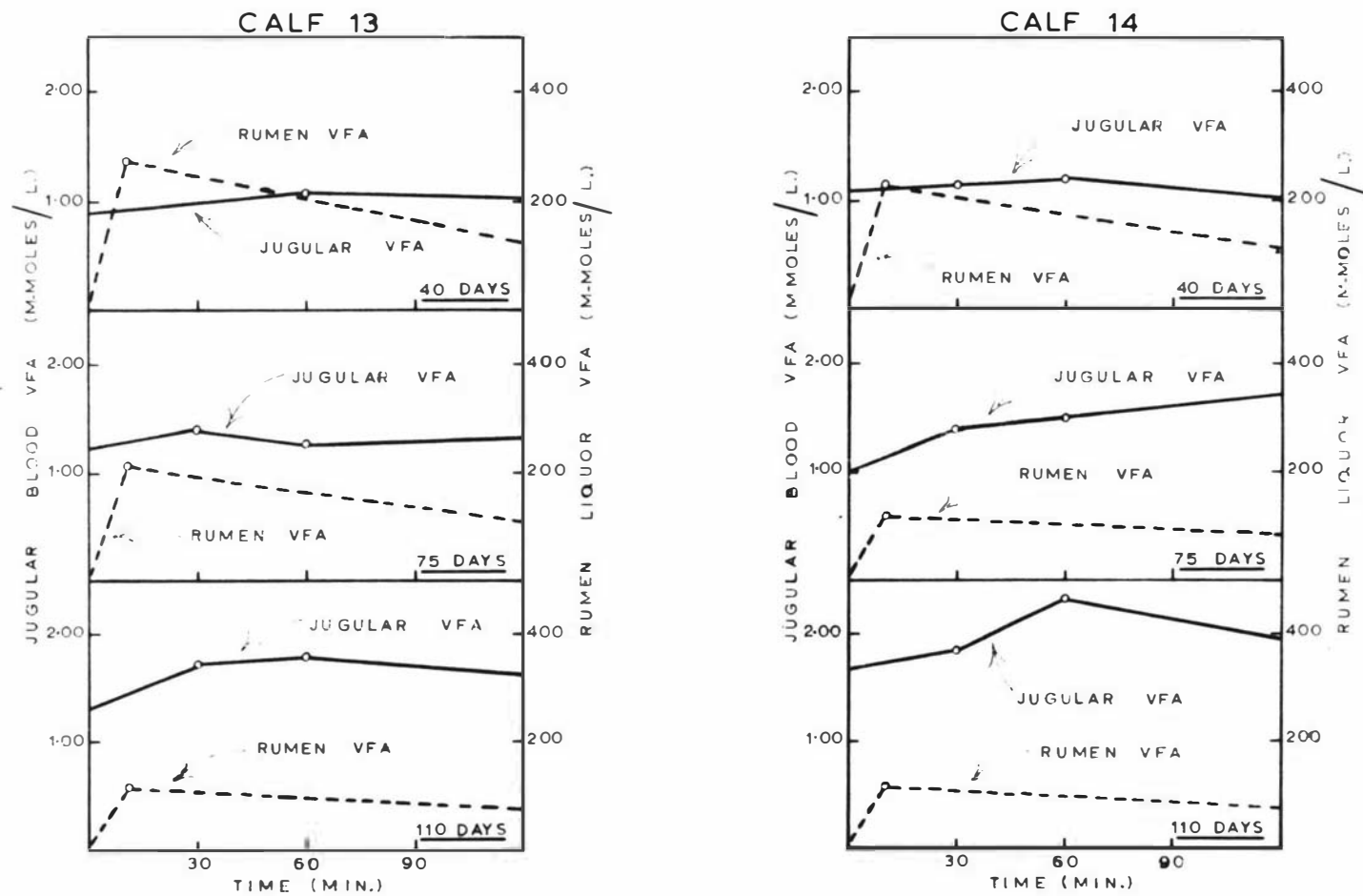


Fig. 20. Changes in the volatile fatty acid concentrations of rumen contents and jugular blood following the introduction of preformed volatile fatty acids into the rumen of the Pasture Fed Calves.

Experiment IV. Absorption of Preformed Volatile Fatty Acids from the Rumen of Conscious Calves Studied by the Analysis of Jugular Blood

Changes in the volatile fatty acid concentration of rumen contents following the introduction of preformed salts of these acids into the rumen (table 29, fig. 19 and 20). The VFA concentration of rumen liquor at the end of the fasting period ranged from 8.84-23.38 m-moles/lit (table 29). There were no apparent fasting concentrations of rumen VFAs that were characteristic of either the age or the diet of the calves.

An increase in the VFA concentration of the rumen was always observed 10 minutes after the buffered VFA solution was introduced into the fore-stomach (table 29, fig. 19 and 20). Although this enhanced concentration was of a similar magnitude in all the calves at the age of 40 days (table 29, fig. 19 and 20), it increased from there on with advancing age in the milk-fed calves (table 29, fig. 19), and decreased in the pasture-fed calves (table 29, fig. 20).

A greater amount of phosphoric acid was required at the age of 40 days to maintain rumen pH between 6.00 and 6.20 in the pasture-fed calves than in the milk-fed calves (table 29). Although the quantity of phosphoric acid needed to control rumen pH increased with advancing age in the pasture-fed calves, it remained much the same in those reared on a diet of milk only (table 29).

Changes in the volatile fatty acid concentration of jugular blood following the introduction of preformed salts of these acids into the rumen (table 29, fig. 19 and 20). The introduction of VFAs into the rumen did not always result in an increase in the concentration of these acids

TABLE 30

Changes in the volatile fatty acid concentration of jugular blood following the injection of a 4 Molar solution containing 85 % acetic and 15 % propionic acid into the jugular vein.

Group	Calf No.	Age days	Weight kg	4N VFA solution administered ml	JUGULAR VFA m-moles/lit						
					0 min	5 min	30 min	45 min	60 min	90 min	120 min
M I	11	35	53	53	1.15	7.28	1.34	1.25	1.42	1.71	-
		70	77	77	1.96	8.77	4.67	2.10	2.03	-	-
		105	110	110	1.55	10.72	3.32	-	1.24	1.72	1.24
L K	12	35	58	58	0.83	7.03	1.66	1.03	1.03	1.20	0.91
		70	88	88	1.93	9.09	4.36	3.71	3.24	-	-
		105	110	110	0.91	8.14	3.20	1.84	2.22	0.96	1.56
P A S T U R E	13	35	61	61	0.94	6.34	1.32	1.20	0.94	0.85	0.97
		70	95	95	0.83	5.69	2.45	1.00	1.25	-	1.45
		105	108	108	1.56	8.31	2.50	1.92	0.99	1.30	1.19
	14	35	59	59	1.05	7.08	1.58	0.83	1.05	0.84	0.74
		70	86	86	0.95	6.16	2.94	1.64	2.00	1.95	1.52
		105	93	93	1.58	10.04	3.81	2.23	2.07	1.55	1.31

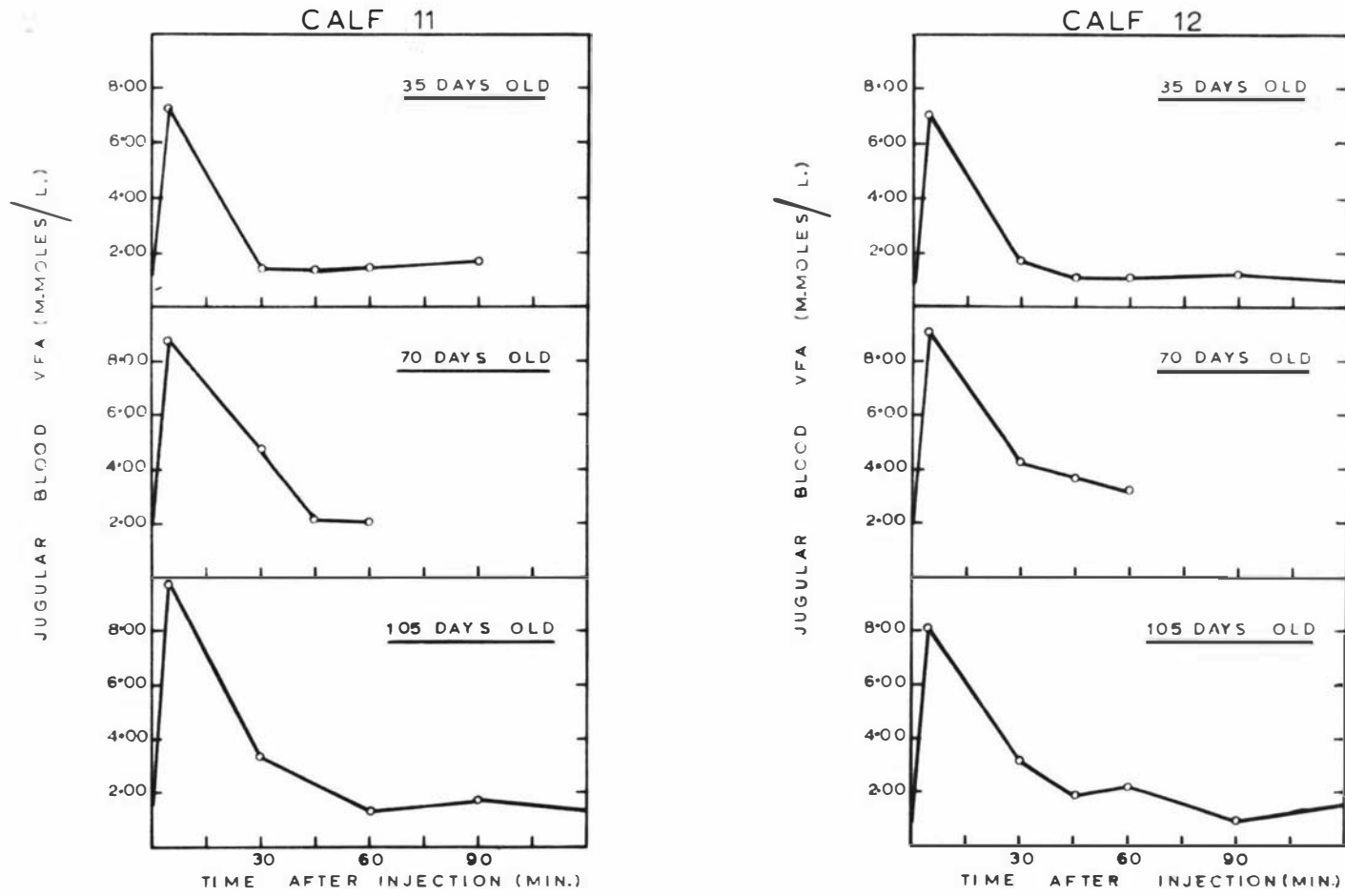


Fig. 21. Changes in the volatile fatty acid concentration of jugular blood following the injection of preformed volatile fatty acids into the jugular vein of the Milk Fed Calves.

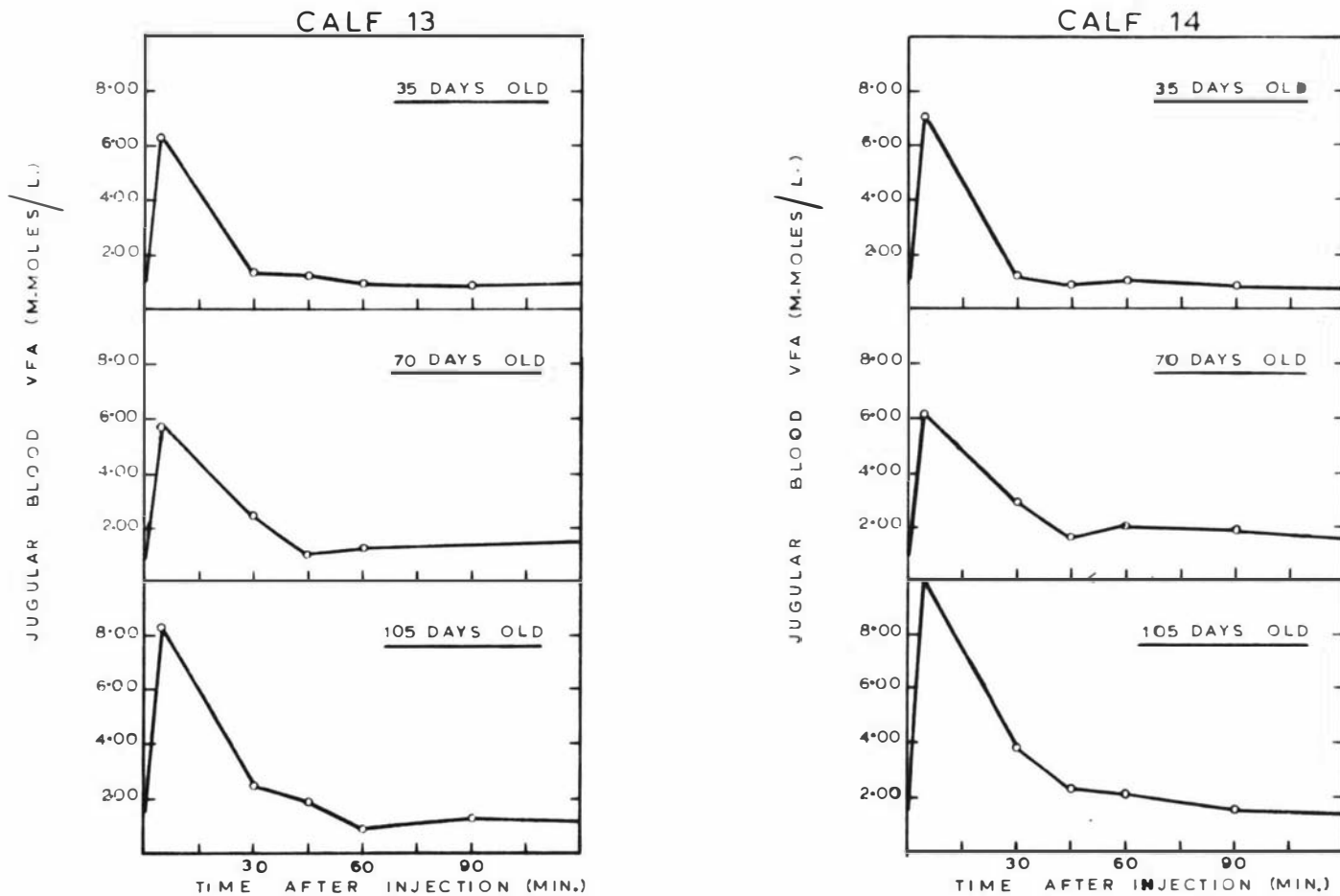


Fig. 22. Changes in the volatile fatty acid concentration of jugular blood following the injection of preformed volatile fatty acids into the jugular vein of the Pasture Fed Calves.

in jugular blood. Enhanced jugular blood VFA levels were noted in calf 12 at the age of 40 and 110 days, in calf 13 at the age of 110 days and in calf 14 at the age of 75 and 110 days (table 29, fig. 19 and 20).

Experiment V. Metabolism of Preformed Volatile Fatty Acids in Conscious Calves Studied by the Analysis of Jugular Blood

Changes in the volatile fatty acid concentration of jugular blood following the injection of preformed salts of these acids into the jugular vein (table 30, fig. 21 and 22). A marked increase in the VFA concentration of jugular blood was invariably observed 5 minutes after the injection of preformed salts of the VFAs into the jugular vein (table 30, fig. 21 and 22). This initial rise was followed by a rapid decline to the pre-injection levels, usually within 45-60 minutes after the administration of the VFAs (table 30, fig. 21 and 22). There was no apparent difference in the clearance of these acids from the peripheral circulation characteristic either of the age of the calves or their diet.

Health and Growth Rate of the Calves (table 25)

The calves were healthy and continued to grow throughout the rearing period (table 25). A few mild cases of nutritional scours were encountered during the initial stages of milk feeding. These scattered incidents were easily arrested by reducing the amount of milk consumed and by sulphaguanidine (Imperial Chemical Industries) medication. Both milk-fed calves developed a craving appetite. At the age of 90 days calf 12 developed an acute case of indigestion and took approximately one week to return back to normal. This was attributed to the consumption of a considerable

quantity of the high pressure plastic hose used for cleaning the pens.

The growth rates of both pairs of calves during the first 10 weeks of age were similar (table 25). Although from then on the milk-fed calves continued to grow at much the same rate as they had maintained before, a marked post-weaning decline in the growth rate of the pasture-fed calves was noted (table 25). The reduced daily gains of calf 12 between the age of 75 and 110 days (table 25) were attributed to indigestion developed at the age of 90 days.

Leakage of rumen contents from around the Jarrett cannulae was not a serious problem in the pasture-fed calves. In these calves the rumen cannulae were lost only occasionally, presumably when they were caught on farm fences. When discovered the rumen fistulae were found to be contracted and it was only after they were lubricated and stretched that the cannulae could be reintroduced. In contrast leakage of rumen contents became a serious problem with advancing age in the milk-fed calves. The rumen fistulae in these calves did not appear to have the same degree of tone as those of the pasture-fed calves. As a result the Jarrett cannulae were frequently dislodged in the milk-fed calves, and had to be reintroduced practically daily during the last 2-3 weeks of the experiment in calf 12. On a number of occasions a partial prolapse of the rumen wall through the fistula was observed when this calf was found lying down with a dislodged cannula.

## DISCUSSION

### Fasting Levels of Volatile Fatty Acids in Jugular Blood (table 28)

A basal fasting level of volatile fatty acids in jugular blood was not apparent in the present study even in the milk-fed calves. Although this finding is in agreement with the observations of Dinda (cited by Preston, 1963), it is not in agreement with those of Martin et al. (1959) who demonstrated fairly reproducible but considerably lower prefeeding concentrations of jugular blood VFAs.

The VFA fraction of jugular blood in adult ruminants has been found to be composed of approximately 15-20 % formic acid, 80-85 % acetic acid and traces of propionic acid (McClymont, 1951; Annison, 1954; Annison et al., 1957). Although these animals derive the bulk of their energy requirements from the VFAs produced in the rumen and caecum (Carroll and Hungate, 1954; Balch, 1958; Blaxter, 1962) almost all the formic acid and a large fraction of the acetic acid (20-40 %) found in jugular blood arise from endogenous sources (Annison, 1954; Annison and White, 1962; Lee and Williams, 1962). The experiments of Kiddle et al. (1951), Pfander and Phillipson (1953), Pennington (1952, 1954) and Annison et al. (1957) have demonstrated the near-complete metabolism of absorbed butyrate by the rumen epithelium. The studies of Schambye (1955 a) and Annison et al. (1957) indicate hepatic removal of most of the propionate and a portion of the acetate assimilated from the alimentary tract.

More recent radio-isotope studies in adult ruminants have revealed the rapid (2-4 minutes) turnover rate of pool acetate (Annison and Lindsay, 1961; Lee and Williams, 1962; Sabine and Conner Johnson, 1964; Conner

Johnson et al., 1965) and the ability of extrahepatic tissues (adipose, kidney, lung, heart and gut) to utilize this acid (Holdsworth, Neville, Nader, Jarrett and Filsell, 1964). Acetyl coenzyme A, the biologically active form of free acetate, is further known to arise from both the anabolism and catabolism of carbohydrates, proteins and lipids. In the light of all these findings it seems difficult to visualize the demonstration of a characteristic basal fasting level of acetate in the blood, as is the case with blood glucose. It would appear to be even more difficult to offer a meaningful interpretation to any deviations from such a level were it demonstrated.

Experiment IV. Absorption of Preformed Volatile Fatty Acids from the Rumen of Conscious Calves Studied by the Analysis of Jugular Blood (table 29, fig. 19 and 20).

The VFA concentration changes of the rumen in the present study (table 29, fig. 19 and 20) cannot be compared because:-

1. The VFAs, although administered on a body weight basis, were nevertheless diluted in whatever liquor was present in the rumen at the time, and thereby created a different set of conditions within the rumen at the beginning of each trial.
2. There were no means of accounting for either the entry of saliva into the rumen or the flux of water across the rumen wall.

The initial induced elevations of rumen VFA concentrations (10 minutes after the introduction of the VFAs into the rumen) did however indicate an increase in the volume of rumen contents relative to body weight with

advancing age in the pasture-fed calves (table 29, fig. 20). This observation is similar to that made by Warner et al. (1956) and Tamate et al. (1962), and is most likely a genuine one, since only minor leakage of rumen contents was encountered in these calves. The apparent decrease in the volume of rumen contents relative to body weight noted in the milk-fed calves with advancing age cannot be considered as genuine since considerable leakage of rumen contents was encountered in these calves.

In describing their technique for the study of VFA absorption from the rumen Sutton et al. (1962) found that "when absorption was studied at pH 6.6, approximately 2 M of phosphoric acid were added to the rumen for each mole of VFA absorbed." This observation would suggest that in the present study a greater amount of VFAs was absorbed by the pasture-fed calves than by the milk-fed calves at the age of 40 days, and that whereas VFA absorption remained much the same with advancing age in the milk-fed calves, it increased considerably in the pasture-fed calves (table 29). Such a conclusion, although in agreement with the later findings of Sutton et al. (1963), does not indicate how pasture intake stimulates VFA absorption: whether solely by an enhanced anatomical development of the rumen relative to other parts of the alimentary tract and body weight, or whether, in addition to this, by the post-natal histological and physiological maturation of the epithelium also.

Despite all the precautions taken to avoid undue exposure of the rumen wall to deleterious stimuli, the introduction of preformed VFAs into the fasted rumen was not always followed by increases in the levels of these acids in jugular blood, not even in the pasture-fed calves, in which the rumen epithelium was presumably capable of VFA assimilation. Rapid

metabolism of absorbed VFAs (Schambye, 1955 a; Annison et al., 1957; Annison and Lindsay, 1961; Lee and Williams, 1962; Sabine and Conner Johnson, 1964; Conner Johnson et al., 1965) may have been responsible for the removal of absorbed VFAs from the peripheral circulation. The results of this experiment demonstrated the unsuitability of jugular blood for the study of VFA absorption from the rumen, and suggested the analysis of rumen effluent blood in future studies. Although these findings are in agreement with those of Sutton et al. (1963), they do not agree with those of Martin et al. (1959) who demonstrated marked post-prandial increases in the VFA concentration of jugular blood following the feeding of either hay and concentrates or a diet containing the salts of the VFAs (page 7).

Experiment V. Metabolism of Preformed Volatile Fatty Acids in Conscious Calves Studied by the Analysis of Jugular Blood (table 30, fig. 21 and 22)

Although such factors as disturbances in carbohydrate metabolism (Pugh and Scarisbrick, 1952; Jarrett and Potter, 1957; Jarrett and Filsell, 1962), fasting (Reid, 1958; Jarrett and Filsell, 1960), and composition of the diet (Reid, 1958; Jarrett and Filsell, 1960; Egan, 1965) were shown to alter acetate tolerance in sheep, the results of the present study in calves indicate that advancing age and rumen development did not affect the clearance of intravenously imposed loads of VFAs from the peripheral circulation. The results suggest that the calves were equally capable of utilizing the VFAs regardless of whether they were reared on milk only or whether they had, in addition to milk, free access to pasture. This observation is in partial agreement with the findings of Young et al. (1964) who demonstrated that milk-fed calves are capable of utilizing large

quantities of VFAs.

### Health and Growth Rate of the Calves

The similar growth rates of the calves up to the age of 40 days (table 25) are not surprising, since both Godfrey (1961) and Stewart (1962) demonstrated that mature VFA levels are first detected in the rumen of pasture-reared calves between the ages of 3 and 4 weeks. The continued similarity in growth rates between the ages of 40 and 75 days (table 25) is unexpected, and suggests that milk and not pasture served as the principal source of nutrients during this period in the pasture-fed calves. This observation indicates that the contributions of the rumen to the general nutrition of pasture-reared calves fed whole-milk at the daily rate of 12.5 % of body weight are of no great moment. Pasture intake, although probably restricted on this high plain of whole-milk feeding, nevertheless appears to exert a substantial stimulatory effect on the functional development of the rumen, witnessed by the positive, although greatly reduced, post-weaning growth rates of the pasture-fed calves (table 25).

The mere demonstration of either mature concentrations of rumen VFAs (Godfrey, 1961; Stewart, 1962) or high digestibility coefficients of solid feeds (Preston et al., 1957; McArthur, 1957) in young calves does not therefore appear to constitute by itself a good criterion for evaluating the contributions of the rumen to the general nutrition of the calf. Although the ingestion of solid feeds and the initiation of fermentation may be the primary stimuli to rumen development (Flatt et al., 1958; Sander et al., 1959; Tamate et al., 1962) the evidence presented

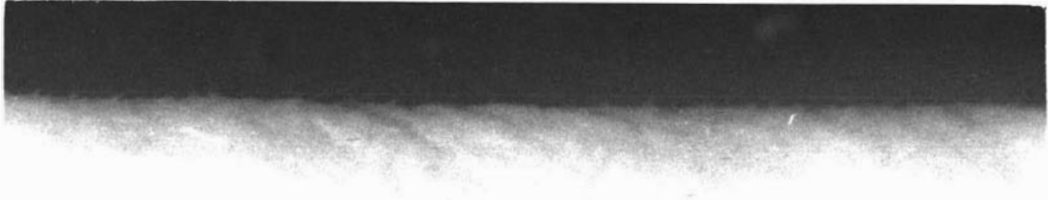


Fig. 23. The rumen wall in a 15-week-old milk-fed calf (Calf No.12).

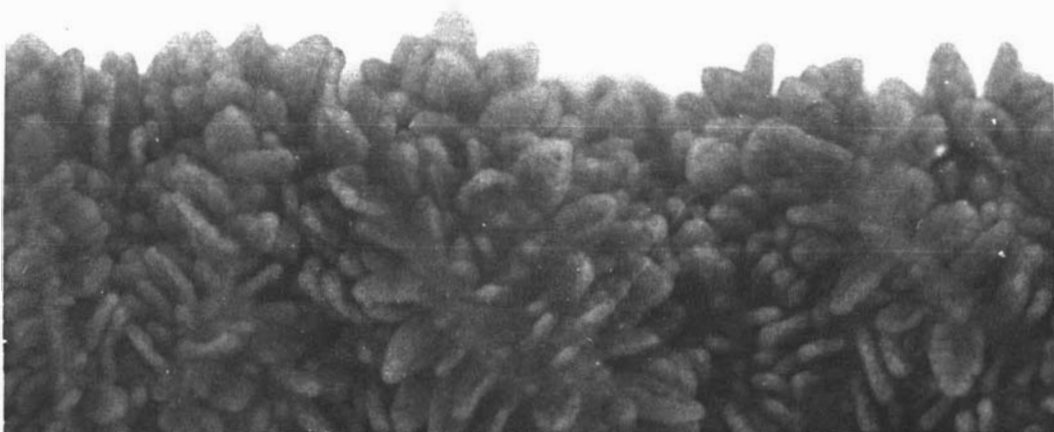


Fig. 24. The rumen wall in a 15-week-old pasture-reared calf (Calf No.14).

in this chapter suggests that the amount of solid feed intake is affected by the level of milk feeding. The ramifications of this observation, as far as pasture-reared calves are concerned, include the interactions of the level and frequency of milk feeding on pasture intake and rumen development.

Autopsy of the calves at the end of the experiment revealed a marked retardation in the development of the rumen wall in the milk-fed calves. The rumen wall at 15 weeks of age was macroscopically still very much the same as that of newly born calves: thin and devoid of papillae (fig. 23). In contrast, the rumen wall of pasture-fed calves resembled that of mature ruminants: thick and highly papillated (fig. 24). These observations are similar to those reported by Flatt et al. (1956) and Brownlee (1956) on milk-fed calves and on calves that received, in addition to milk, hay and concentrate mixtures. These findings suggest that the problem of leaking fistulae and dislodged cannulae in the milk-fed calves was more than likely a consequence of the retarded development of the rumen wall.

#### SUMMARY

1. Volatile fatty acid absorption and metabolism tests were conducted at regular intervals with advancing age in two pairs of Friesian bull-calves, one pair of which was reared on milk only (Milk Fed Calves), and the other pair on milk and continuous access to pasture from the age of 10 days onwards (Pasture Fed Calves).
2. Absorption was studied by the appearance of VFAs in the jugular blood following the introduction of these acids into the rumen. Metabolism

was investigated by the clearance of intravenously imposed loads of VFAs from the peripheral circulation.

3. A basal fasting level of jugular blood VFAs characteristic of either the age or diet of the calves was not apparent in these studies.
4. The experiments demonstrated:-
  - (a) the unsuitability of jugular blood for the study of VFA absorption from the rumen of calves;
  - (b) that the utilization of VFAs by calves was unaffected by either advancing age or diet.
5. The experiments suggested the analysis of rumen effluent blood in future VFA absorption studies.

S E C T I O N I I

VOLATILE FATTY ACID ABSORPTION STUDIES

I N

ANAESTHETIZED CALVES

## CHAPTER IV

### CHANGES IN THE VENO-ARTERIAL VOLATILE FATTY ACID CONCENTRATION DIFFERENCE OF RUMINAL BLOOD FOLLOWING THE INTRODUCTION OF PREFORMED VOLATILE FATTY ACIDS INTO THE RUMEN OF ANAESTHETIZED CALVES

The volatile fatty acid absorption studies in conscious calves described in Section I of this Thesis (Chapters I, II and III) demonstrated:

1. The complications associated with the chronic catheterization of the portal vein.
2. The presence of endogenous sources of circulating VFAs.
3. The rapid removal of absorbed VFAs from the circulation.
4. The absence of a characteristic basal fasting level of circulating VFAs.

These studies further suggested that the effects of advancing age and diet on VFA absorption would be better investigated in young calves by changes in the veno-arterial VFA concentration difference of the rumen following the introduction of the preformed salts of these acids into the rumen. The problems associated with the collection of rumen effluent and arterial blood samples from calves within a few days after birth, however, limited the application of this experimental approach to acute preparations.

REVIEW OF LITERATURE

Volatile Fatty Acid Absorption Studies in Mature Anaesthetized Ruminants

Indirect evidence of VFA absorption from the rumen was first presented by Phillipson and McAnally (1942). These workers investigated the VFA concentration within the compound stomach of mature anaesthetized sheep and demonstrated:-

- (a) that the VFA concentration was higher in the rumen than in the abomasum;
- (b) that the introduction of the sodium salts of the VFAs into the rumen did not increase the concentration of these acids in the abomasum;
- (c) that the VFAs were stable when incubated in rumen liquor.

On the basis of these observations they concluded that the VFAs were absorbed from the alimentary tract before reaching the abomasum.

More definitive proof of VFA absorption from the rumen of mature anaesthetized sheep taken straight from pasture was presented by McAnally and Phillipson (1942), who demonstrated that the VFA concentration of rumen effluent blood was higher (1.7-7.1 m-moles/lit) than that of either intestinal or peripheral blood (0.1-0.9 m-moles/lit). They also introduced equimolar solutions of the sodium salts of the VFAs into the emptied and washed rumen and noted that the rate of VFA absorption was a function of the molecular weight of these acids, being fastest for acetate and slowest for butyrate. These findings were confirmed by Barcroft et al. (1944) who also observed that the VFA content of both rumen liquor and rumen effluent blood was mainly composed of acetic acid, and that the proportion of the higher molecular weight VFAs (propionate and butyrate)

was greater in the rumen than in the blood. No effort was made, in either of these two series of experiments, to control rumen pH, which had previously been observed by Phillipson (1942) to remain relatively stable and rarely to exceed the limits of 5.8-7.5.

Danielli et al. (1946), Gray (1948) and Masson and Phillipson (1951) investigated the effects of pH on VFA absorption from the isolated washed rumen of anaesthetized sheep. Although there was some difference in their findings concerning the amounts and rates at which the individual fatty acids were absorbed, they all agreed that the magnitude of VFA absorption was greater under the slightly acidic conditions characteristic of the rumen than at alkaline pHs.

Masson and Phillipson (1951) introduced equimolar solutions of the individual VFAs into the isolated washed rumen of anaesthetized sheep and demonstrated that acetate produced the greatest veno-arterial VFA concentration difference in the circulation of the rumen, and that this difference was related to the concentration of the acids in the rumen. Their experiments also indicated that the appearance of these acids in rumen effluent blood was not proportional to the rates at which they disappeared from the rumen.

The latter observation was confirmed by Kiddle, Marshall and Phillipson (1951) and Pfander and Phillipson (1953). It suggested the metabolism of absorbed VFAs by the rumen epithelium and led to the investigations of Pennington and his co-workers (Pennington, 1952, 1954; Pennington and Sutherland, 1955), who showed, in a series of both in vitro and in vivo experiments, that the rumen epithelium does indeed metabolize the VFAs, and that its activity is greatest with butyrate and least with acetate.

TABLE 31

The ages, birth weights and weight gains of the calves used in the acute experiments.

Experimental Particulars	G R O U P I Milk			G R O U P II Milk & Pasture			G R O U P III Milk		
	1	2	3	4	5	6	7	8	9
Age at time of experiment, days	5	5	3	40	41	44	42	42	43
Birth weight, kg	36	30	39	37	38	37	28	35	33
Weight at time of experiment, kg	38	32	39	70	70	67	46	45	44
*Rate of gain, kg/day	-	-	-	0.82	0.78	0.68	0.43	0.24	0.25

\*Average rate of gain/group, kg/day: Group II 0.76

Group III 0.32

## Volatile Fatty Acid Absorption Studies in Young Anaesthetized Ruminants

No reference was found in the literature consulted of any published work on the absorption of VFAs from the rumen of young anaesthetized milk-fed calves.

### MATERIALS AND METHODS

#### Experimental Animals

Nine Friesian bull-calves were randomly assigned to one of three experimental groups following 2 days of colostrum feeding on their dams (table 31). Group I and Group III calves were fed on a diet of milk only and were denied access to solid feeds. These calves were confined to concrete pens and yards where slatted wooden frames constituted their only bedding. Group II calves were reared, from the age of 5 days onwards, on pasture - a mixture of white clover, short-rotation and long-rotation rye grass - . Warm, whole Jersey milk was bucket-fed twice daily to all the calves at the rate of 4.5 lit/calf/day, irrespective of age or treatment. The birth weights, final weights, growth rates and ages of the calves are presented in table 31.

#### Experimental Design

The acute VFA absorption experiments described in this chapter were performed at the age of 3-5 days in Group I calves, and at the age of 40-44 days in the other two groups (table 31). These experiments involved the introduction of a buffered solution of preformed VFAs into the rumen, and the simultaneous sampling of both arterial and rumen effluent blood at



Fig. 25. Entry into the abdominal cavity.



Fig. 26. Rumen cannula in position.

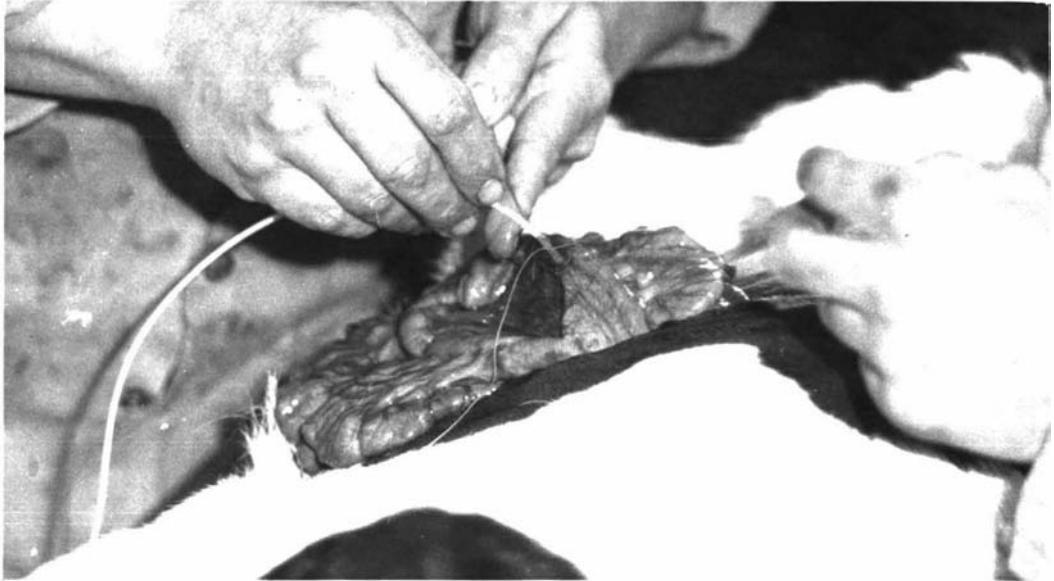


Fig. 27. Catheterization of mesenteric vein radical.

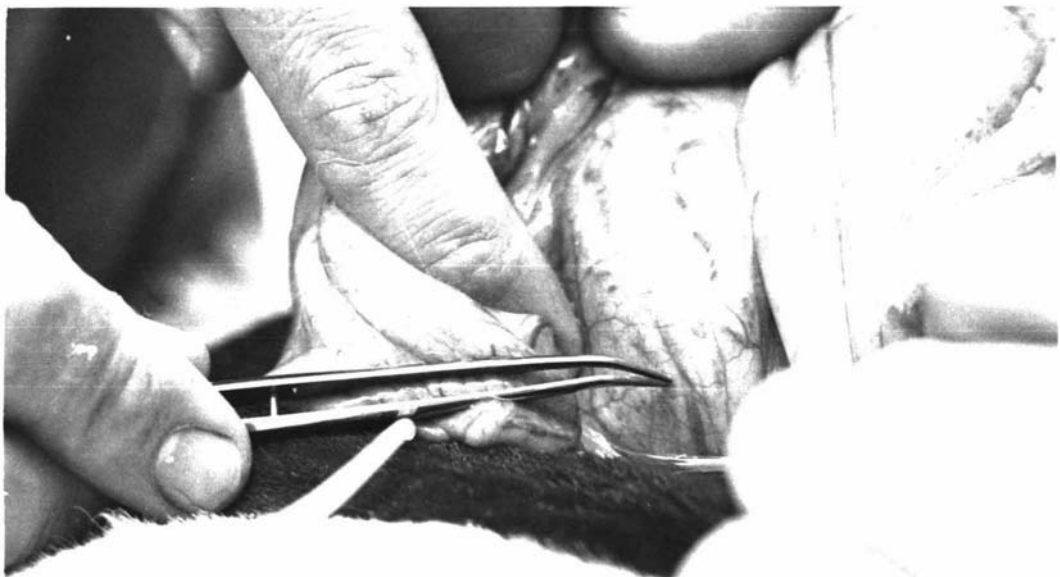


Fig. 28. The right ruminal vein.

regular intervals thereafter. Solutions were introduced into the rumen through a rumen cannula. A stomach tube was used to empty the rumen. The left carotid artery and the right ruminal vein served as sources of arterial and rumen effluent blood respectively.

#### Surgical Preparation of the Calves.

The calves were fasted prior to the experiments. Milk was withheld for a period of 17-20 hours. Group II calves were removed from pasture 45-50 hours before they were to be surgically prepared for the absorption experiments, and were kept in concrete calf yards.

Anaesthesia was induced and maintained by the intravenous injection of 900-1500 mg of pentobarbitone sodium (Nembutal, Abbott Laboratories). An 8-12 cm midventral neck incision was made to cannulate the trachea and expose the left carotid artery.

Entry into the abdominal cavity was gained from the left side through a 15-20 cm skin incision (fig. 25). This incision ran along the last intercostal space in Group I and Group III calves, and along the anterior border of the left paralumbar fossa in Group II calves. The last two ribs were resected in Group I and Group III calves to expose the rumen. A glass cannula (i.d. 1 cm) was inserted into the posterior sac of the rumen and retained in position with a purse-string suture (fig. 26). The mesenteric vein was catheterized with Teflon tubing (i.d. 1.5 mm) through one of its radicals (fig. 27). This catheter was connected to an infusion apparatus through which a 0.9 % NaCl drip was maintained.

The right ruminal vein on the medial aspect of the rumen was exposed and cannulated (fig. 28). Polythene tubing (i.d. 1 mm) was used in Group I

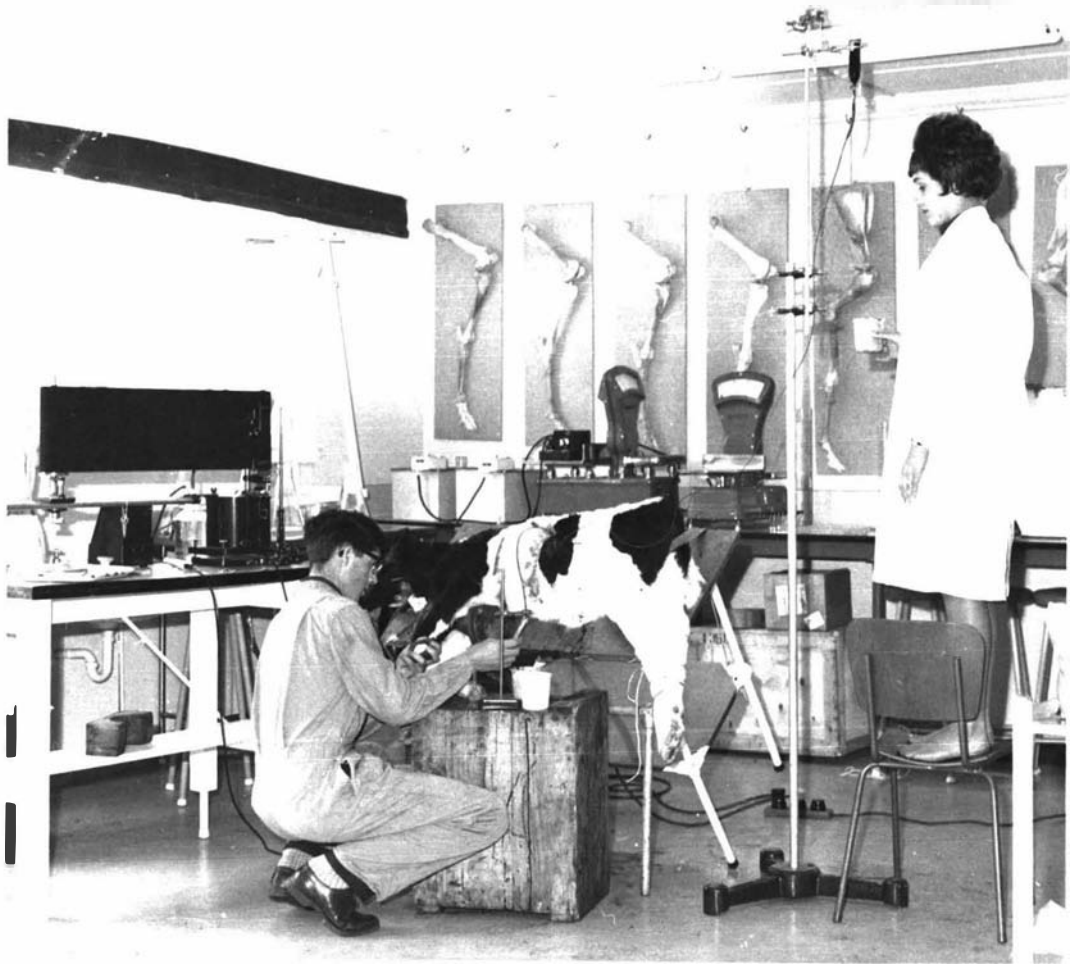


Fig. 29. An absorption experiment in progress.

calves. The difficulties encountered in diverting the normal flow of blood through an angle of  $180^{\circ}$  led to the design of a U-shaped venous glass cannula. This cannula, when connected to wide-bore Teflon tubing (i.d. 3 mm), proved to be more satisfactory than the polythene catheters, and was used in all subsequent experiments. Immediately after the catheter was inserted into the right ruminal vein, heparin (Pularin, Evans Medical) was administered through the mesenteric vein catheter in a dose of 1000 i.u./kg of body weight.

A stomach tube was introduced into the rumen through the mouth. The calves were then strapped to the operating table which was tilted to an angle of approximately  $40^{\circ}$  from the vertical (fig. 29). Finally, the exposed left carotid artery was cannulated and connected to a mercury manometer for both blood pressure recording and arterial blood sampling. Excess rumen effluent blood and arterial blood were returned to the portal circulation via the infusion apparatus.

### Experimental Procedure

The rumen was emptied and rinsed out with warm ( $37^{\circ}\text{C}$ ) 0.9 % NaCl at the beginning of each experiment. Saline was introduced into the rumen through the glass cannula until the level of rumen contents reached the neck of the cannula. The washings were then siphoned out through the stomach tube. This procedure was repeated 5-6 times. An estimate of rumen capacity was obtained by recording the volume of saline that was required to fill the rumen.

Each experiment was composed of three separate 30-minute test periods (unless otherwise stated, fig. 30). Periods A and C served as controls.

A warm (37°C) 0.9 % NaCl solution was introduced into the rumen at the beginning of these periods. The rumen was filled with a warmed (37°C) buffered solution of VFAs at the beginning of Period B. At the end of this period the rumen was again rinsed out with warm (37°C) 0.9 % NaCl (5-6 times). A schematic diagram of this experimental procedure is presented in figure 30.

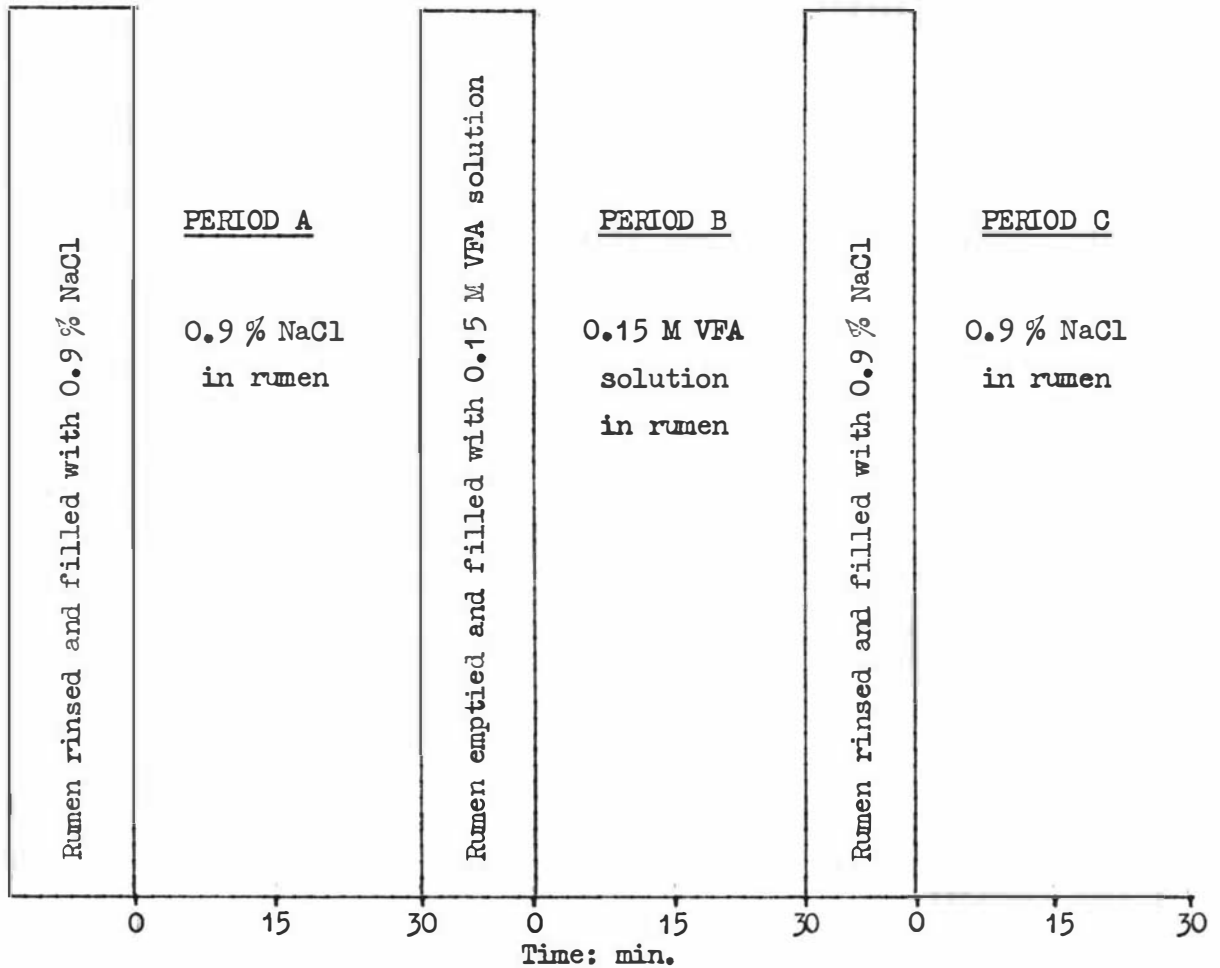


Fig. 30. Schematic diagram of the experimental procedure used in the acute volatile fatty acid absorption experiments.

Simultaneous samples of rumen effluent and carotid blood were collected for VFA analysis 15 and 30 minutes after the test solutions were introduced into the rumen. The rumen was sampled at the beginning (after the introduction of test solutions) and at the end of each period. A plastic wash-



Fig. 31. Outline of venous network in the rumen wall following the injection of methylene blue (Calf No. 8).

bottle was used to aspirate rumen contents. The pH of these samples was determined within one minute of sampling. A Beckman Model 76 pH meter was used. Blood flow through the cannulated right ruminal vein was measured at 10-minute intervals. A graduated glass cylinder was used to estimate the volume of blood collected over a period of one minute. The time-course of the sampling schedule is presented in table 32.

TABLE 32

Time-course of the sampling schedule followed during the acute volatile fatty acid absorption experiments.

Sample	Time after the introduction of test solutions into the rumen min
Right ruminal vein	15 & 30
Left carotid artery	15 & 30
Rumen contents	0 & 30
Blood flow through right ruminal vein	0, 10, 20 & 30

The calves were sacrificed at the end of the experiment. They were bled out through the cannulated left carotid artery. A clear outline of the venous network in the rumen wall following the injection of an aqueous solution of methylene blue through the right ruminal vein catheter was considered sufficient evidence for the proper location of the catheter (fig. 31).

Preparation of the Buffered Volatile Fatty Acid Solution (table 33)

A 0.15 M solution of VFAs containing 70 % acetic acid, 25 % propionic acid and 5 % butyric acid was made up in Krebs Ringer and Sorensen's phosphate buffer (Sutton et al., 1962). The pH of this solution was adjusted to 6.00 with 4 N NaOH. Twenty-five lit, the composition of which is

presented in table 33, were prepared in the following way:-

The fatty acids (B.D.H., Analar) were diluted in 2 lit of distilled water and titrated to a pH of 6.00 with 4 N NaOH (B.D.H., Analar). Krebs Ringer and Sorensen's phosphate buffer components (B.D.H., Analar) were dissolved in another 2 lit of distilled water and added to the VFA solution. The volume was made up to 25 lit with distilled water and the pH was again adjusted to 6.00 by the addition of 2 ml of 4 N NaOH. This solution was stored at a temperature of 4°C and was used in all the experiments described in this chapter.

TABLE 33

Composition of 25 lit of volatile fatty acid rumen infusion

Component	Proportion per cent	Molarity	Quantity gm
<u>Volatile Fatty Acids</u>		0.15	
Acetic acid	70	-	157.6
Propionic acid	25	-	69.4
Butyric acid	5	-	16.5
<u>Krebs Ringer Solution</u>			
Potassium chloride (KCl)			9.3187
Calcium chloride (CaCl <sub>2</sub> .6H <sub>2</sub> O)			16.4317
Magnesium sulphate (MgSO <sub>4</sub> .7H <sub>2</sub> O)			3.4022
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )			6.1625
<u>Sorensen's Phosphate Buffer</u>		0.01	
Disodium hydrogen phosphate (Na <sub>2</sub> HPO <sub>4</sub> .2H <sub>2</sub> O)			5.3440
Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )			2.9955

#### Chemical Methods

Annison's (1954) method as modified - the details and accuracy of which were described earlier - was used for the determination of blood VFAs.

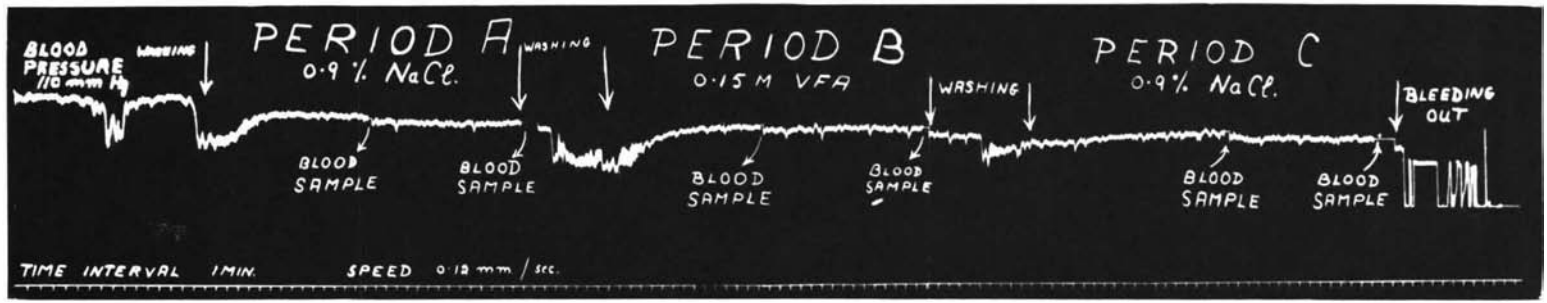


Fig. 32. Recording of blood pressure taken during one of the absorption experiments.

TABLE 34

Volatile fatty acid concentration of blood collected simultaneously from the right ruminal vein and carotid artery

Experimental Particulars			VFA Concentration of Blood (m-moles/lit)								
			G R O U P I Milk			G R O U P II Milk & Pasture			G R O U P III Milk		
Calf No.			1	2	3	4	5	6	7	8	9
P E R I O D A 0.9 % NaCl	Ruminal vein	15 min	2.43	1.15	3.29	1.52	1.75	2.12	1.90	2.71	2.32
		30 "	-	-	-	2.39	2.93	2.53	1.92	2.27	2.02
		45 "	1.73	1.11	2.20	-	-	-	-	-	-
	Carotid artery	15 min	1.56	1.13	2.10	1.50	3.05	1.82	1.47	2.67	2.22
		30 "	-	-	-	2.39	3.51	2.35	1.87	2.63	2.18
		45 "	1.78	1.41	2.14	-	-	-	-	-	-
P E R I O D B 0.15 M VFA	Ruminal vein	15 min	4.29	2.52	3.14	3.75	1.69	4.57	2.75	4.71	5.09
		30 "	3.66	2.20	5.47	4.86	4.69	3.96	2.77	4.57	5.33
	Carotid artery	15 min	1.98	1.30	2.59	3.71	1.48	2.46	1.96	2.81	2.48
		30 min	2.36	1.52	2.75	2.52	2.17	2.53	1.77	2.57	2.86
P E R I O D C 0.9 % NaCl	Ruminal vein	15 min	2.41	1.54	-	2.52	1.96	2.34	1.93	3.05	3.44
		30 "	1.66	1.71	-	2.63	1.22	2.28	1.63	2.85	2.06
	Carotid artery	15 min	1.53	1.86	-	2.13	1.77	2.50	1.76	3.05	3.82
		30 "	1.81	1.39	-	2.63	1.71	2.15	2.09	3.14	3.56

TABLE 35

Volatile fatty acid concentration of arterial carotid blood for different groups in different periods. Samples taken 15 minutes after the introduction of test solutions into the rumen.

(See appendix table 47 for analysis of variance)

Experimental Particulars	VFA Concentration of Carotid Blood m-moles/lit			Period Means m-moles/lit
	GROUP I	GROUP II	GROUP III	
PERIOD A 0.9 % NaCl	1.60	2.12	2.12	1.95
PERIOD B 0.15 M VFA	1.96	2.55	2.42	2.31
PERIOD C 0.9 % NaCl	1.98*	2.13	2.88	2.33
Group Means	1.85	2.27	2.47	

\*Contains one 'missing plot' (Snedecor, 1956).

Period and group means are averages for 9 calves.

Figures in body of table are averages for 3 calves.

Standard error of individual mean: 0.39 m-moles/lit.

Significance of differences:

Between group means: Not significant at the 10 % level.

Between period means: Not significant at the 10 % level.

TABLE 36

Volatile fatty acid concentration of arterial carotid blood for different groups in different periods. Samples taken 30 minutes after the introduction of test solutions into the rumen.

(See appendix table 48 for analysis of variance)

Experimental Particulars	VFA Concentration of Carotid Blood m-moles/lit			Period Means m-moles/lit
	GROUP I	GROUP II	GROUP III	
PERIOD A 0.9 % NaCl	1.78	2.75	2.23	2.25
PERIOD B 0.15 M VFA	2.21	2.41	2.40	2.34
PERIOD C 0.9 % NaCl	1.83*	2.16	2.93	2.30
Group Means	1.94	2.44	2.52	

\*Contains one 'missing Plot' (Snedecor, 1956).

Period and group means are averages for 9 calves.

Figures in body of table are averages for 3 calves.

Standard error of individual means: 0.23 m-moles/lit.

Significance of differences:

Between group means: Not significant at the 10 % level.

Between period means: Not significant at the 10 % level.

TABLE 37

Veno-arterial volatile fatty acid concentration differences of blood collected simultaneously from the ruminal vein and carotid artery

Experimental Particulars		Veno-Arterial VFA Concentration Differences of the Rumen (m-moles/lit)								
		G R O U P I Milk			G R O U P II Milk & Pasture			G R O U P III Milk		
Calf No.		1	2	3	4	5	6	7	8	9
P E R I O D A 0.9 % NaCl	15 min	+0.87	+0.02	+1.19	+0.02	-1.30	+0.30	+0.43	+0.04	+0.10
	30 "	-	-	-	0.00	-0.58	+0.18	+0.05	-0.36	-0.16
	45 "	-0.05	-0.30	+0.06	-	-	-	-	-	-
P E R I O D B 0.15 M VFA	15 min	+2.31	+1.22	+0.55	+0.04	+0.21	+2.11	+0.79	+1.90	+2.61
	30 "	+1.30	+0.68	+2.72	+2.34	+2.52	+1.43	+1.00	+2.00	+2.47
P E R I O D C 0.9 % NaCl	15 min	+0.88	-0.32	-	+0.39	+0.19	-0.16	+0.17	0.00	-0.38
	30 "	-0.15	+0.32	-	0.00	-0.49	+0.13	-0.46	-0.29	-1.50

TABLE 38

Veno-arterial volatile fatty acid concentration differences for different groups in different periods. Samples taken 15 minutes after the introduction of test solutions into the rumen.

(See appendix table 49 for analysis of variance)

Experimental Particulars	Veno-arterial VFA Concentration Differences m-moles/lit*			Period Means m-moles/lit
	GROUP I	GROUP II	GROUP III	
PERIOD A 0.9 % NaCl	2.69	1.67	2.19	2.18
PERIOD B 0.15 M VFA	3.36	2.79	3.77	3.31
PERIOD C 0.9 % NaCl	2.20**	2.14	1.93	2.09
Group Means	2.75	2.20	2.63	

\*Data coded, m-moles/lit + 2

\*\*Contains one 'missing plot' (Snedecor, 1956).

Period and group means are averages for 9 calves.

Figures in body of table are averages for 3 calves.

Significance of differences:

Between group means: Not significant at the 10 % level.

Between period means:  $(P_B \text{ v } \frac{P_A + P_C}{2})$  Significant at the 1 % level.

Interaction between periods and groups: Not significant at the 10 % level.

TABLE 39

Veno-arterial volatile fatty acid concentration differences for different groups in different periods. Samples taken 30 minutes after the introduction of test solutions into the rumen.

(See appendix table 50 for analysis of variance)

Experimental Particulars	Veno-arterial VFA Concentration Differences m-moles/lit*			Period Means m-moles/lit
	GROUP I	GROUP II	GROUP III	
PERIOD A 0.9 % NaCl	1.90	1.87	1.84	1.87
PERIOD B 0.15 M VFA	3.57	4.10	3.82	3.83
PERIOD C 0.9 % NaCl	2.41**	1.88	1.25	1.85
Group Means	2.63	2.62	2.30	

\*Data coded, m-moles/lit + 2

\*\*Contains one 'missing plot' (Snedecor, 1956).

Period and group means are averages for 9 calves.

Figures in body of table are averages for 3 calves.

Significance of differences:

Between group means: Not significant at the 10 % level.

Between period means:  $(F_B \text{ v } \frac{P_A + P_C}{2})$  Significant at the 1 % level.

Interaction between periods and groups: Not significant at the 10 % level.

## RESULTS

The results of the acute VFA absorption experiments are presented in tables 34, 37, 40, 41, 42 and 43. In the case of calves 1, 2 and 3, the delay in sampling during Period A was caused by the displacement of the right ruminal vein cannula, and the time taken to recatheterize the vein. At the end of Period B in the case of calf 3 the right ruminal venous catheter was found to be no longer patent and the experiment was terminated.

Blood pressure remained relatively constant throughout the duration of any one experiment (fig. 32). Fluctuations in blood pressure were noted when solutions were introduced into the rumen and when the stomach tube was manipulated (fig. 32).

### Absorption of Volatile Fatty Acids from the Rumen (tables 34, 35, 36, 37, 38 and 39).

Volatile fatty acid absorption from the rumen was demonstrated in all nine calves irrespective of their age or diet (table 37). The veno-arterial VFA concentration differences of the rumen were greater following the introduction of VFAs into the rumen (Period B) than in either of the two control periods (Periods A and C) when saline was introduced. Statistical analysis of the experimental data (tables 35, 36, 38 and 39) showed that:-

1. The veno-arterial VFA concentration differences were significantly higher (at the 1 % level) following the introduction of VFAs into the rumen (Period B) than during the control periods (Periods A and C).

TABLE 40

Blood flow rates through the right ruminal vein catheter during acute volatile fatty acid absorption experiments.

Experimental Particulars		Blood Flow Rate Through Right Ruminal Vein Catheter ml/min								
		G R O U P I Milk			G R O U P II Milk & Pasture			G R O U P III Milk		
Calf No.		1	2	3	4	5	6	7	8	9
P E R I O D A 0.9 % NaCl	0 min	6.5	9.5	13.0	28.0	22.0	20.0	15.0	30.0	34.0
	10 "	6.5	-	11.5	26.0	20.0	46.0	18.0	28.0	32.0
	20 "	4.5	-	13.0	22.0	33.0	30.0	18.0	28.0	27.0
	30 "	-	11.0	-	16.0	40.0	32.0	18.0	33.0	23.0
	40 "	4.0	9.0	7.0	-	-	-	-	-	-
P E R I O D B 0.15 M VFA	0 min	4.0	10.2	4.5	13.0	32.0	22.0	17.0	26.0	22.0
	10 "	4.0	11.0	5.0	17.0	38.0	30.0	18.0	20.0	22.0
	20 "	4.5	11.0	4.5	15.0	30.0	20.0	16.0	30.0	18.0
	30 "	3.0	11.0	4.5	14.0	24.0	26.0	16.0	22.0	18.0
P E R I O D C 0.9 % NaCl	0 min	4.5	9.0	-	12.0	22.0	26.0	17.0	20.0	16.0
	10 "	3.0	10.5	-	8.0	26.0	26.0	19.0	21.0	16.0
	20 "	3.0	12.0	-	8.0	20.0	19.0	10.0	22.0	12.0
	30 "	3.0	13.0	-	8.0	20.0	20.0	8.0	22.0	14.0

TABLE 41

Mean blood flow rates through the right ruminal vein catheter.  
(See appendix table 51 for analysis of variance)

Experimental Particulars	Blood Flow Rate Through Right Ruminal Vein Catheter ml/min								
	G R O U P I Milk			G R O U P II Milk & Pasture			G R O U P III Milk		
Calf No.	1	2	3	4	5	6	7	8	9
Mean flow rate of individual calves, Period A	5.4	9.8	11.1	23.0	28.7	32.0	17.2	29.7	29.0
" " " " " " Period B	3.9	10.8	4.6	14.7	31.0	24.5	16.7	24.5	20.0
" " " " " " Period C	3.4	11.1	-	9.0	22.2	22.7	13.5	21.2	14.5
" " " " " " Entire experiment	4.2	10.6	7.8	15.6	27.2	26.4	15.8	25.1	21.2
Mean flow rate of calf groups Period A	8.8			27.9			25.3		
" " " " " " Period B	6.4			23.4			20.4		
" " " " " " Period C	7.2			18.0			16.4		
" " " " " " Entire experiment	7.5			23.1			20.7		

Significance of differences:

Between group means: Significant at the 5 % level.

Between period means:  $(P_B \text{ v } \frac{P_A + P_C}{2})$  Not significant at the 10 % level.

Between periods:  $(P_A \text{ v } P_C)$  Significant at the 1 % level.

2. The changes in the veno-arterial VFA concentration difference following the introduction of VFAs into the rumen (Period B) were of a similar magnitude in the three groups of calves, and did not differ significantly. (The interaction between periods and groups was not significant at the 10 % level).
3. The three groups of calves did not differ greatly in the veno-arterial VFA concentration differences of the rumen during any one of the three test periods. (The difference between groups as well as the interaction between groups and periods was not significant at the 10 % level).

A basal fasting level of circulating VFAs characteristic of either the age or the diet of the calves was not apparent in these anaesthetized animals. The VFA concentration of both arterial blood and rumen effluent blood fluctuated during the control periods (Periods A and C) of any one experiment (table 34).

#### Blood Flow Through the Right Ruminal Vein Catheter (tables 40 and 41).

Fluctuations in blood flow through the right ruminal vein catheter were noted in all the calves in practically every test period (table 40). The mechanical factors involved in diverting the normal flow of blood through an angle of  $180^{\circ}$  were found to be at least in part responsible for these fluctuations, since minor reorientation of the catheters frequently resulted in profound changes in blood flow. Statistical analysis of the mean blood flow rates (table 41) recorded during the different test periods indicated that:-

TABLE 42

Changes in the pH of rumen contents during the acute volatile fatty acid absorption experiments

Experimental Particulars		pH of Rumen Contents								
		G R O U P I Milk			G R O U P II Milk & Pasture			G R O U P III Milk		
Calf No.		1	2	3	4	5	6	7	8	9
P E R I O D A 0.9 % NaCl	0 min	7.16	6.23	6.57	7.21	7.32	6.98	6.64	6.62	6.90
	30 "	-	-	-	7.11	7.02	6.94	6.60	6.50	6.75
	45 "	6.95	5.90	6.11	-	-	-	-	-	-
P E R I O D B 0.15 M VFA	0 min	6.10	6.05	6.07	6.23	6.21	6.15	6.08	6.08	6.12
	30 "	6.51	6.25	6.38	6.47	6.53	6.52	6.30	6.31	6.35
P E R I O D C 0.9 % NaCl	0 min	7.70	6.06	6.18	6.86	6.88	6.66	6.34	6.37	6.65
	30 "	6.59	6.95	5.68	6.79	6.86	6.76	6.51	6.50	6.74

1. The difference in the rate of blood flow through the right ruminal vein catheter between the younger calves (Group I) and the older ones (Group II and III) was significant (at the 5 % level).
2. The difference in the rate of blood flow through the right ruminal vein catheter between the two groups of older calves (Group II and Group III) was not significant (at the 10 % level).
3. The introduction of VFAs into the rumen (Period B) did not result in a significant (not significant at the 10 % level) increase in the rate of blood flow through the right ruminal vein catheter in any one of the three groups of calves.
4. The decrease in blood flow through the right ruminal vein catheter between the first (Period A) and the second (Period C) control periods was highly significant (at the 1 % level).

#### pH of Rumen Contents (table 42)

An increase in the pH of the rumen was noted in all the calves following the introduction of VFAs into the rumen (Period B, table 42). The magnitude of this increase was not characteristic of any one group of calves.

A decrease in the pH of the rumen was recorded in all the calves during the first control period (Period A, table 42). The pH of the rumen increased during the second control period (Period C) in calves 2, 6, 7, 8 and 9, and decreased in the others (table 42).

TABLE 43

## Rumen capacity of experimental calves

Experimental Particulars	G R O U P I Milk			G R O U P II Milk & Pasture			G R O U P III Milk		
	1	2	3	4	5	6	7	8	9
VFA solution introduced, ml	500	500	500	2000	2000	2000	1200	1300	1500
*Rumen capacity, ml/kg body weight	13.1	15.6	12.8	28.6	28.6	29.8	26.1	28.9	34.1

\*Average rumen capacity/kg live weight/group, ml: Group I 13.8

Group II 29.0

Group III 29.7

### Rumen Capacity (table 43)

For comparative purposes the estimates of rumen capacity were expressed in ml/kg of live body weight (table 43). Rumen capacity ranged from 12.8-15.6 ml/kg of body weight in Group I calves, 28.6-29.8 ml/kg of body weight in Group II calves and 26.1-34.1 ml/kg of body weight in Group III calves (table 43).

### Growth Rate of the Calves (table 31)

The birth weight of the calves ranged from 28-39 kg (table 31). All the calves were healthy throughout the rearing period and none were observed to suffer from any of the calfhood diseases. The growth rate of the calves raised on pasture was higher than that of the calves reared on milk only. Group II calves gained an average of 0.76 kg/calf/day, and Group III 0.31 kg/calf/day (table 31).

## DISCUSSION

Volatile fatty acid absorption from the rumen was first demonstrated in anaesthetized animals (McAnally and Phillipson, 1942). For a number of years following this initial observation acute preparations served as the major source of information concerning VFA transfer across the rumen wall (Barcroft et al., 1944; Danielli et al., 1946; Gray, 1948; Masson and Phillipson, 1951; Kiddle et al., 1951; Pfander and Phillipson, 1953) since the investigation of this phenomenon in conscious animals had to await the development of techniques for the chronic catheterization of the portal vein (Schanbye and Phillipson, 1949; Annison et al., 1957).

Although acute experiments represent a major departure from the normal physiological conditions that prevail in the intact animal many of the qualitative aspects of VFA transport made in anaesthetized preparations were later confirmed in conscious animals. More recent experiments indicate that anaesthesia, surgery, loss of blood, starvation and washing of the rumen all retard the rate of VFA absorption (Schambye, 1955 a & b; Dobson and Phillipson, 1956; Annison et al., 1957; Fegler and Hill, 1958; Bensadoun and Reid, 1962; Sutton et al., 1963).

Absorption of Volatile Fatty Acids from the Rumen (tables 34, 35, 36, 37, 38 and 39)

Evidence of VFA absorption from the rumen of 3-5 day-old calves (Group I) was presented in the results of the acute experiments described in this chapter (table 37). The ability to absorb VFAs appears to be inherent in the rumen wall and does not seem to be dependent on either the consumption of solid feeds or the establishment within the rumen of a microbial population that is characteristic of the mature animal. Such a finding is not surprising since all the evidence to-date indicates that the VFAs are transported across the rumen wall by a process of simple diffusion (Dobson, 1961; Annison, 1965). A similar observation was made by Sutton et al. (1963) who investigated the same problem in conscious calves by the disappearance of the VFAs from the rumen rather than by their appearance in the blood (page 10).

The ability of 3-5 day-old calves to metabolize VFAs is also indicated in the present study by the substantial removal of absorbed VFAs from the peripheral circulation (table 34). Young et al. (1964) found that young milk-fed calves are capable of metabolizing the VFAs readily. The above-mentioned two observations, namely the absorption and metabolism of VFAs

by 3-5 day-old calves were later confirmed in another series of acute experiments (Chapter V) in which labelled acetate was incorporated in the buffered VFA solution introduced into the rumen.

Experiments with mature ruminants indicate that the VFAs are transported across the rumen wall by a process of passive absorption, and that the rates of transfer are dependent on the concentration gradients of these acids and on the pH of the rumen (Masson and Phillipson, 1951; Dobson, 1961; Annison, 1965). An increase in the rate of VFA absorption must accompany the post-natal anatomical maturation of the rumen since papillary development leads not only to an expansion of the absorptive surface of the rumen (Warner et al., 1956; Tamate et al., 1962) but also to a greater vascularization of the rumen wall (Dobson et al., 1956).

The acute experiments described in this chapter did not reveal any difference in the rate of VFA transport between 40-44 day-old milk-fed calves (Group III) and calves of the same age which had, in addition to milk, free access to pasture (Group II). Both the blood flow rates through the catheterized right ruminal vein (table 41) and the veno-arterial VFA concentration differences of the rumen (table 37) were of a similar magnitude in these two groups of calves. Sutton et al. (1963) noted an increase in the disappearance of the VFAs from the rumen with advancing age in conscious calves that had been fed on milk, alfalfa pellets and finely ground calf starter, but not in calves that had been reared on a diet of milk only. In the present study the similar absorption rates noted in the two groups of older calves (Group II and Group III) suggest that the rumen papillae were not sufficiently developed in the 40-44 day-old calves that had access to pasture (Group II) to materially increase the rate of

VFA absorption. Evidence that this may have been the case is presented in the experiments of Brownlee (1956) who observed a more rapid development of rumen papillae in milk and concentrate-fed calves than in calves fed on milk and grass.

Endogenous sources of circulating VFAs (Annison and White, 1962; Lee and Williams, 1962) were indicated in this study by the presence of these acids in both rumen effluent and carotid blood during the two control periods (Periods A and C, table 34). Although the circulating levels of VFAs recorded in these two periods (Periods A and C, table 34) were higher than those normally found in fasted conscious animals (Annison et al., 1957), the veno-arterial VFA concentration differences of the rumen were observed to be negligible (table 37). Whether the elevated concentrations of circulating VFAs were caused by the mobilization of body fat during these periods of simulated acute starvation (Periods A and C), or whether they were a result of the administration of heparin, was not determined. The changing concentration of circulating VFAs noted in the control periods (Periods A and C) of any one experiment (table 34) suggested that the contribution of endogenous VFAs to the circulation takes place, at least in acute preparations, at an uneven and irregular rate. Under these conditions the demonstration of a basal fasting level of circulating VFAs would be almost impossible.

#### Blood Flow Through the Right Ruminal Vein Catheter (tables 40 and 41)

Dobson and Phillipson (1956) demonstrated an increase in the rate of blood flow through the rumen of anaesthetized sheep following either the introduction of VFAs into the rumen or the lowering of rumen pH when VFAs

were present. An enhanced level of VFAs in the rumen of conscious animals has also been shown to result in an increase in the rate of blood flow through the portal vein (Bensadoun and Reid, 1962), and the right ruminal artery (Sellers, 1965). The introduction of VFAs into the rumen in the present study (Period B) did not result in a significant increase in blood flow through the right ruminal vein catheter (tables 40 and 41). Mechanical factors associated with the diversion of blood through an angle of  $180^{\circ}$  may have masked an increase in blood flow. The possibility that calves under the age of 40-44 days have not yet developed the capacity or the mechanisms to respond to the addition of VFAs to the rumen in a similar fashion as do older animals must also be taken into account.

A greater rate of blood flow through the right ruminal venous catheter was noted in the older calves (Group II and Group III) than in the younger ones (Group I). This increase was most probably characteristic of the older animals since similar rates of blood flow were observed in both groups of older calves (Group II and Group III). The decreased flow rates recorded during the second (Period C) of the two control periods (Periods A and C) may have been a reflection of a gradual deterioration of the preparations.

#### pH of Rumen Contents (table 42)

Danielli et al. (1946) and Sutton et al. (1962) demonstrated an increase in the pH of rumen contents following the introduction of VFAs into the isolated and washed rumen, and attributed this rise in pH to the absorption of VFAs from the rumen. The studies of Masson and Phillipson (1951) and Ash and Dobson (1963) showed that the transfer of VFAs across

the rumen wall was accompanied by the entry of bicarbonate into the rumen.

In the present study the introduction of VFAs into the rumen was always followed by an increase in rumen pH (Period B, table 42). Although this finding does not by itself constitute evidence of VFA transport across the rumen wall, it nevertheless is in agreement with the observations of Danielli et al. (1946), Masson and Phillipson (1951), Sutton et al. (1962) and Ash and Dobson (1963).

#### Rumen Capacity (table 43)

The effects of advancing age and diet on rumen capacity were investigated in Friesian calves by Warner et al. (1956) and Tamate et al. (1962). They slaughtered calves at various ages and estimated rumen volume by aqueous distention to a constant head. Rumen volume in new-born calves was found to range between 0.5 and 1.6 lit. The rumen in concentrate and hay-fed calves reached adult proportions (relative to body weight and other parts of the compound stomach) by the age of 13-15 weeks. Although Tamate and his co-workers noted an increase in rumen volume relative to body weight with advancing age in milk-fed calves, Warner and his colleagues did not make a similar observation. Smith (1959) estimated the volume of rumen contents in conscious calves by the introduction of polyethylene glycol into the rumen of milk-fed calves. His estimates of rumen contents were less than the distended rumen volumes determined by Warner et al. (1956) and Tamate et al. (1962). He did, however, demonstrate a progressive increase in the volume of rumen contents relative to body weight with advancing age in milk-fed calves.

In the present study the distended rumen volume of the calves which

had access to pasture (Group II) was greater than that of the calves fed on a diet of milk only (Group III, table 43). When expressed on a body-weight basis, these estimates of distended rumen volumes were however comparable for both groups of older calves (Group II and Group III). An increase in the distended volume of the rumen relative to body weight was noted with advancing age in the calves fed on a diet of milk only (Group III); this is in agreement with the findings of both Smith (1959) and Tamate et al. (1962). Free access to pasture up to the age of 40-44 days (Group II) did not appear to stimulate a detectable increase in the distended rumen volume determined in these calves under anaesthesia.

#### Growth Rate of the Calves (table 31)

Although both groups of older calves (Group II and Group III) were fed the same daily amounts of milk, the growth rate of the calves that had access to pasture (Group II) was more than twice that of the calves that were fed on milk only (Group III). This greater growth rate must at least in part be attributed to the consumption of pasture since the milk-fed calves (Group III) did not appear to suffer from any of the calfhood diseases. The growth rates noted in the present study indicate that calves under the age of 40-44 days can utilize pasture; presumably this is an indication of the establishment at quite an early age of an effective rumen fermentation. It has been recorded by both Godfrey (1961) and Stewart (1962) that adult VFA concentrations can be detected in the rumen of 3-4 week-old pasture-reared calves.

SUMMARY

1. The effects of advancing age and diet on VFA absorption from the rumen were studied in anaesthetized calves by changes in the veno-arterial VFA concentration difference of the rumen following the introduction of VFAs into the rumen.
2. Three groups of calves were used. Group I and Group III calves were reared on a diet of milk only. Group II calves had, in addition to milk, free access to pasture from the age of 5 days onwards.
3. Acute VFA absorption experiments were performed at the age of 3-5 days in Group I calves and at the age of 40-44 days in Group II and Group III calves.
4. Increases in the veno-arterial VFA concentration of the rumen were noted in 3-5 day-old calves following the introduction of the VFAs into the rumen.
5. Volatile fatty acid utilization following absorption in the 3-5 day-old calves was indicated by the degree of removal of these acids from the peripheral circulation.
6. No differences in the rate of VFA absorption from the rumen were apparent between 40-44 day-old milk-fed calves and calves of a similar age which, in addition to milk, had free access to pasture.
7. These studies suggested the use of radio-active isotopes in further acute experiments.

## CHAPTER V

### THE APPEARANCE OF ACETATE-1-C<sup>14</sup> IN RUMEN EFFLUENT AND CAROTID BLOOD FOLLOWING THE INTRODUCTION OF PREFORMED VOLATILE FATTY ACIDS INTO THE RUMEN OF ANAESTHETIZED CALVES

Transfer of VFAs across the rumen wall was demonstrated in anaesthetized 3-5 day-old calves by changes in the veno-arterial VFA concentration differences of the rumen (Chapter IV). Rapid removal of absorbed VFAs from the peripheral circulation was also indicated in these studies.

The acute experiments described in this chapter were aimed at obtaining confirmation of the above-mentioned two observations. Radio-active acetate was added to the VFA solution introduced into the rumen, and its appearance in the blood was considered as evidence of absorption.

#### REVIEW OF LITERATURE

No reference was found in the literature consulted to work with radio-active isotopes on the absorption of VFAs from the rumen of conscious or anaesthetized calves during the first week of life.

## MATERIALS AND METHODS

### Experimental Animals

Three Friesian bull-calves were used. The calves were transferred to concrete calf-pens after two days of oolostrum feeding on their dams. Details of their age and body weight at the time of the experiments are presented in table 44.

TABLE 44

The age and body weight of the calves.

Calf No.	Age days	Body weight kg
10	4	35
11	3	33
12	4	34

### Experimental Design

The calves were 3-4 days old when the experiments were performed (table 44). They were fasted for a period of 24 hours prior to the experiments. Anaesthesia and surgical procedures were similar to those described in Chapter IV.

### Experimental Procedure

The rumen was emptied and rinsed with warm (37°C) 0.9 % NaCl at the beginning of each experiment, after which 0.5 lit of the buffered VFA solution (Chapter IV) containing 150  $\mu$  curies of acetate-1-C<sup>14</sup> (Radiochemical Centre, Amersham, England) were introduced into the rumen through the glass cannula. Simultaneous samples of rumen effluent and carotid

TABLE 45

Volatile fatty acid concentration and radio-activity of blood collected simultaneously from the right ruminal vein and carotid artery.

Experimental Particulars		VFA Concentration of Blood m-moles/lit			Radio-activity of Blood counts/min (corrected for background)		
		Calf 10	Calf 11	Calf 12	Calf 10	Calf 11	Calf 12
Ruminal vein	0 min	3.12	3.23	2.96	1	0	0
	15 "	4.23	3.38	4.19	86	20	38
	30 "	4.02	4.18	3.92	110	26	44
	45 "	4.54	3.94	-	139	40	-
	60 "	4.62	3.41	-	153	59	-
Carotid artery	0 min	2.74	3.12	2.97	0	0	0
	15 "	3.35	3.02	3.61	5	0	1
	30 "	3.75	3.19	3.49	7	0	2
	45 "	3.38	3.44	-	4	0	-
	60 "	4.05	3.17	-	5	0	-

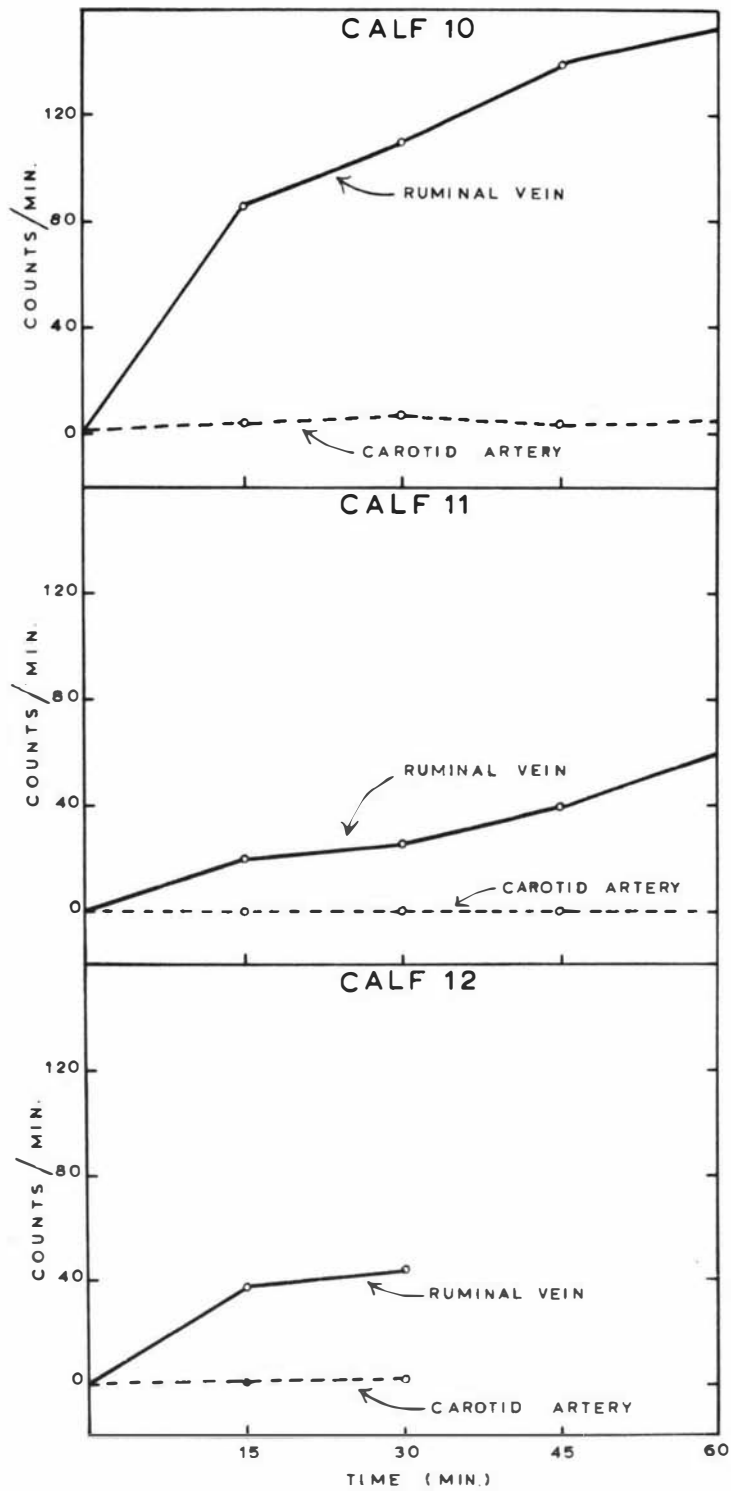


Fig. 33. Radio-activity of rumen effluent and carotid blood samples.

blood were collected 0, 15, 30, 45 and 60 minutes after the introduction of the test solution into the rumen. These samples were later analysed for their VFA concentration by the method described in Chapter I (Annison, 1954). Blood flow through the right ruminal venous catheter was measured 0, 10, 20, 30, 40, 50 and 60 minutes after the introduction of the VFA solution into the rumen.

The titrated steam distillates of blood were evaporated to dryness (80°C) after the addition of a further 0.2 ml of 0.01 N NaOH. They were then reconstituted in 1 ml of distilled water. Aliquots of 0.1 ml were transferred onto glass planchettes and dried under an infra-red lamp. Radioactivity was determined by Geiger-Müller detection using both a Phillips FW 4141 Gas-Flow Detector and a Phillips 18506 mica end-window detector (standard error 4 %).

## RESULTS

Tables 45 and 46 summarize the results of the acute experiments described in this chapter. The ruminal vein catheter lost its patency within half an hour in the case of calf 12 and the experiment was terminated.

Transfer of acetate-1-C<sup>14</sup> across the rumen wall was demonstrated in each of the three calves studied (table 45, fig. 33). The rate of acetate-1-C<sup>14</sup> absorption appeared to increase as the experiments progressed (table 45, fig. 33). Rapid metabolism of absorbed acetate-1-C<sup>14</sup> was indicated by the near-complete removal of the isotope from the peripheral circulation (table 45, fig. 33).

TABLE 4.6

Blood flow rates through the right ruminal vein catheter during the acute volatile fatty acid absorption experiments in which radio-active acetate was introduced into the rumen.

Time after the introduction of VFAs into the rumen	Blood Flow Rate Through the Right Ruminal Vein Catheter ml/min		
	Calf 10	Calf 11	Calf 12
0 min	12.4	12.0	11.0
10 "	16.0	17.5	15.5
20 "	14.5	14.0	9.0
30 "	14.5	13.0	4.5
40 "	12.5	9.0	-
50 "	10.0	-	-
60 "	10.0	4.0	-

An increase in the flow of blood through the right ruminal vein catheter was observed in all three calves 10 minutes after the VFAs were introduced into the rumen (table 46). Following this initial rise, a gradual decline in blood flow was noted as the experiments progressed.

#### DISCUSSION

Unequivocal evidence of acetate- $1-C^{14}$  transfer across the rumen wall in 3-4 day-old calves was presented in the acute experiments described in this chapter by the appearance of the isotope in rumen effluent blood (table 45, fig. 33). Whether the increasing rate of acetate- $1-C^{14}$  transport noted in all three experiments as they progressed was a function of the time taken for the isotope to diffuse across the rumen wall, or whether it indicated an active form of absorption was not determined. Regardless of whether the assimilation of acetate- $1-C^{14}$  was by active or passive processes, the results of the present study indicated that the ability to absorb the isotope from the rumen was not a consequence of either the consumption of solid feeds or the establishment within the rumen of a microbial population that is characteristic of an adult ruminant. The increasing capacity to absorb VFAs that accompanies rumen development seems therefore to be dependent on the expansion of pre-existing mechanisms rather than on the development of new ones.

The increasing concentration of acetate- $1-C^{14}$  in rumen effluent blood noted in all the experiments as they progressed was not accompanied by a similar rise in the radio-activity of carotid blood. Although dilution of rumen effluent blood in the peripheral circulation could have contributed in part to the low radio-activity of carotid blood, this near-complete

removal of acetate-1-C<sup>14</sup> from the peripheral circulation suggests the rapid metabolism of the isotope by the 3-4 day-old anaesthetized calves used in this study.

Dobson and Phillipson (1956), Bensadoun and Reid (1962) and Sellers (1965) have demonstrated that an enhanced level of VFAs in the rumen of adult animals results in an increased rate of blood flow through the fore-stomach. A similar initial rise in blood flow was noted in the present study following the introduction of the buffered VFA solution into the rumen of the 3-4 day-old anaesthetized calves. The gradual decline in blood flow observed following the initial rise (table 46) might have been due to the deterioration of the preparations during the course of these experiments.

#### SUMMARY

1. Volatile fatty acid transfer across the rumen wall was studied in three anaesthetized 3-4 day-old Friesian bull-calves by the appearance of acetate-1-C<sup>14</sup> in rumen effluent blood following its introduction into the rumen.
2. Absorption of acetate-1-C<sup>14</sup> was demonstrated in all three calves.
3. The rate of acetate-1-C<sup>14</sup> transfer increased as the experiments progressed.
4. Rapid metabolism of absorbed acetate-1-C<sup>14</sup> was indicated by the near-complete removal of the isotope from the peripheral circulation.

## CONCLUSION

The results of the studies described in this Thesis indicate that calves are capable of absorbing VFAs from the rumen and of utilizing these acids during the first week of post-natal life, prior to the consumption of solid feeds and the establishment within the rumen of a microbial fermentation similar to that found in adult animals.

On the basis of these observations it would appear that the utilization of solid feeds by calves during early post-natal life is not limited by their inability to absorb or metabolize the VFAs that are produced in the rumen by microbial fermentation. The limiting factor to solid feed utilization seems to be one of intake, since all the evidence to date indicates that the initiation of a microbial fermentation within the rumen of young calves reared on pasture which has been recently grazed by older ruminants is a natural consequence of solid feed intake.

Whether or not calves can be induced to consume solid feeds sooner and in greater quantities than they normally do by limiting milk intake and improving the palatability of solid feeds are matters for future investigation.

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Appendix

TABLE 47

Analysis of variance of the volatile fatty acid concentration of arterial carotid blood for different groups in different periods. Samples taken 15 minutes after the introduction of test solutions into the rumen.

Source of Variance	Degrees of Freedom	Mean Square	F
Total	25	-	-
Periods	2	0.4513	Not significant
Periods x calves within groups	11	0.4579	
Periods x groups	4	0.1701	Not significant
Groups	2	0.8387	Not significant
Calves within groups	6	0.7493	

TABLE 48

Analysis of variance of the volatile fatty acid concentration of arterial carotid blood for different groups in different periods. Samples taken 30 minutes after the introduction of test solutions into the rumen.

Source of Variance	Degrees of Freedom	Mean Square	F
Total	25	-	-
Periods	2	0.0347	Not significant
Periods x calves within groups	11	0.1566	
Periods x groups	4	0.4461	Not significant
Groups	2	0.5754	Not significant
Calves within groups	6	0.6553	

TABLE 49

Analysis of variance of the veno-arterial volatile fatty acid concentration differences for different groups in different periods. Samples taken 15 minutes after the introduction of test solutions into the rumen.

Source of Variance	Degrees of Freedom	Mean Square	F
Total	25	-	-
Periods	2	4.1038	P < 1 %
P <sub>B</sub> v $\frac{P_A + P_C}{2}$	1	8.1667	P < 1 %
P <sub>A</sub> v P <sub>C</sub>	1	0.0410	Not significant
Periods x calves within groups	11	0.5168	
Periods x groups	4	0.4070	Not significant
Groups	2	0.7539	Not significant
Calves within groups	6	0.5948	

TABLE 50

Analysis of variance of the veno-arterial volatile fatty acid concentration differences for different groups in different periods. Samples taken 30 minutes after the introduction of test solutions into the rumen.

Source of Variance	Degrees of Freedom	Mean Square	F
Total	25	-	-
Periods	2	11.6374	P < 1 %
P <sub>B</sub> v $\frac{P_A + P_C}{2}$	1	23.2723	P < 1 %
P <sub>A</sub> v P <sub>C</sub>	1	0.0025	Not significant
Periods x calves within groups	11	0.3971	
Periods x groups	4	0.4000	Not significant
Groups	2	0.4307	Not significant
Calves within groups	6	0.3228	

TABLE 51

Analysis of variance of the mean blood flow rates through the right ruminal vein catheter\*

Source of Variance	Degrees of Freedom	Mean Square	F
Total	25	-	-
Periods	2	0.073504	
$P_B$ v $\frac{P_A + P_C}{2}$	1	0.003366	Not significant
$P_A$ v $P_C$	1	0.143643	$P < 1 \%$
Periods x calves within groups	11	0.008860	
Groups	2	0.728670	$P < 5 \%$
Calves within groups	6	0.074194	

\*Data transformed to logarithms for analysis of variance to eliminate a correlation between the variance and mean.