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Ion transport across rumen and omasum epithelium

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The interior of the rumen in cattle and sheep is normally maintained at a potential of about −40 mV relative to the blood. This potential depends primarily on the occurrence of an active transport of sodium from rumen to blood, since the potential, short-circuit current and the net sodium flux are simultaneously abolished by anoxia, ouabain and removal of sodium from the bathing solutions. There is an appreciable net flux of potassium from blood to rumen. There is also a substantial active transport of chloride in the same direction as sodium and it can be reduced by treatment with acetazolamide without affecting the potential or the sodium system. Nevertheless, sodium transport is reduced by the removal of chloride ions.

Omasum epithelium is similar to rumen epithelium. However, the chloride pump appears to work in both directions in this tissue. Short-circuited omasum epithelium can also transport magnesium from omasum to blood.

Introduction

The reticulum, rumen and omasum comprise the first three parts of the complex four-compartment stomach of ruminant animals such as cattle and sheep. These compartments are lined with a squamous stratified epithelium which is keratinized and non-glandular. The abomasum or fourth compartment is lined with a glandular epithelium which secretes hydrochloric acid and pepsin and is comparable to the gastric mucosa of animals with simple stomachs. The reticulum and rumen together form the large functional unit which contains the bacterial and protozoan organisms responsible for fermentation of the dietary plant material ingested. The omasum is positioned between the reticulo-rumen and abomasum and may be partly concerned with controlling the flow of digesta into the abomasum. It is a relatively small compact organ with a large internal surface area produced by many longitudinal laminae or leaves which project into the body of the organ.

The microbial digestion of plant food produces large amounts of the short-chain volatile fatty acids, acetic acid, propionic acid and butyric acid, which are known to be absorbed across the rumen wall in considerable quantities (see review by Dobson & Phillipson 1968). To buffer the products of fermentation in the rumen there is a large daily secretion of saliva, and this process assists in the maintenance of the appropriate conditions for the growth of the symbiotic population of rumen micro-organisms. It is now recognized that the reticulo-rumen and omasum are important organs for the absorption of short-chain fatty acids, water and electrolytes. Some of these, such as sodium, must undergo almost complete internal recycling in conditions where the dietary supply of these ions is low.

The electrical potential difference

Dobson & Phillipson (1958) reported that the contents of the rumen in the sheep are electrically negative by about 30 mV with respect to the blood. This observation was confirmed by Ferreira, Harrison, Keynes & Nauss (1966*a*) who also recorded similar potentials across the

omasum with an electrode introduced between two laminae in an anaesthetized sheep. In experiments on conscious sheep involving a change in diet from hay to fresh grass, Sellars & Dobson (1960) reported a positive correlation between the potential and rumen [K^+] and these observations were extended to sodium-depleted sheep by Scott (1966) who found a linear–logarithmic relation between potential and rumen [K^+], with a mean slope of 43 mV for a ten-fold change in [K^+]. This finding supported the observations made by Harrison, Keynes & Nauss (1964) and Ferreira *et al.* (1966*a*) in experiments on anaesthetized sheep with the reticulo-rumen isolated so that solutions of differing composition could be introduced into the rumen. When [Na^+] was kept constant and [K^+] increased or decreased, the potential was observed to vary linearly with log [K^+], with a slope of about 40 mV for a tenfold concentration change. With [K^+] kept constant the potential was directly proportional to [Na^+] and the slope was approximately 0.2 mV $mmol^{-1}\,l^{-1}$. The solutions were made up with sulphate anions to reduce the possible shorting of the potential by chloride ions. When chloride was substituted for sulphate, both the slope and the absolute size of the potential were slightly reduced.

Because of the limitations in the use of anaesthetized sheep, methods were developed for working with isolated sheets of rumen epithelium from which the muscle layers had been stripped (Ferreira, Harrison & Keynes 1964, 1966*b*; Stevens 1964). When the epithelium was immediately mounted and bathed with a sheep Ringer solution containing the substrates glucose, acetate, propionate and butyrate, a reasonably stable preparation was obtained which maintained a good electrical potential and short-circuit current (s.c.c.) for 4 to 6 h. In experiments where the [K^+] was varied on either the blood or the rumen side of the epithelium with [Na^+] kept constant, both sides responded like potassium electrodes with the electrical potential linearly related to log [K^+]. With constant [K^+], there was little or no response of the potential to changes in [Na^+] between 20 and 100 mmol/l on either side of the epithelium (Ferreira *et al.* 1966*b*). Changing the chloride-ion concentration on either side of the epithelium produced only small changes in electrical potential (Harrison, Keynes & Zurich 1968) and did not support the view of Dobson & Phillipson (1958) that chloride might be a freely diffusing ion in this tissue.

In spite of the evidence obtained in conscious and anaesthetized sheep and with isolated epithelium that the passive permeability of K^+ ions may make an appreciable contribution to the total potential generated across the rumen, the primary potential is dependent on the presence of a sodium concentration greater than 12 mmol/l in the bathing solutions (Ferreira *et al.* 1966*b*). The primary potential is abolished by the exclusion of Na, by anoxia and by the addition of ouabain to the blood side of the epithelium, and it seems reasonable to conclude that this potential is produced by the 'active' transport of sodium found in later experiments.

Cation fluxes

In 1959 Dobson presented good evidence for the existence of a sodium pump capable of transporting sodium against its electrochemical potential gradient from the rumen to the blood in anaesthetized sheep. By applying the techniques of Ussing & Zerahn (1951) we have made simultaneous measurements of ion fluxes and short-circuit current with rumen and omasum epithelium. We have usually used two sets of apparatus and mounted adjacent pieces of epithelium from the same sheep so that unidirectional measurements of isotope fluxes could be made from lumen to blood in one preparation and blood to lumen in the other. Our early observations were made in the presence of sulphate as the major anion and in the absence of

chloride ions. These measurements showed fairly large fluxes of Na in both directions which, in two 30 min periods during the third hour after adding the isotope (^{22}Na) labelled Ringer, represented mean values of 1.64 μmol cm^{-2} h^{-1} from rumen to blood and 0.54 μmol cm^{-2} h^{-1} from blood to rumen, or a mean net flux from rumen to blood of 1.10 μmol cm^{-2} h^{-1}. The corresponding s.c.c. was 0.56 μmol cm^{-2} h^{-1} which could account for only 51 % of the Na current. In similar experiments with ^{42}K, there was a flux of K from rumen to blood of 0.07 μmol cm^{-2} h^{-1} in the third hour after adding isotope and a flux of 0.35 μmol cm^{-2} h^{-1} from blood to rumen, indicating an active transport of K from blood to rumen of 0.28 μmol cm^{-2} h^{-1} which would account for 26 % of the total Na current found earlier. The mean s.c.c. in this group of experiments was 0.61 μmol cm^{-2} h^{-1} (56 % of Na current). Experiments using chloride-based Ringer solutions indicated larger fluxes of Na in both directions (3rd hour mean data: 2.85 μmol cm^{-2} h^{-1} from rumen to blood; 1.28 μmol cm^{-2} h^{-1} from blood to rumen; ten paired experiments) and a larger net flux from rumen to blood of 1.57 μmol cm^{-2} h^{-1}. The mean s.c.c. was 0.46 μmol cm^{-2} h^{-1} which could account for only 29 % of the Na current. Potassium fluxes determined in the third hour in three paired experiments gave values of 0.18 μmol cm^{-2} h^{-1} from rumen to blood and 0.54 μmol cm^{-2} h^{-1} from blood to rumen. The net flux of K (0.36 μmol cm^{-2} h^{-1}) in these experiments was 23 % of the total Na current measured in the previous group of experiments, though the s.c.c. was slightly larger in the K experiments (0.58 μmol cm^{-2} h^{-1} or 37 % of the Na current; cf. 0.46 in Na experiments).

Experiments with omasum epithelium indicated larger electrical potentials and short-circuit currents. Measurements of Na flux in the second hour of three paired experiments (for omasum experiments 'paired' usually means adjacent halves of the same leaf) indicated a flux from omasum to blood of 5.23 μmol cm^{-2} h^{-1} compared with 2.07 μmol cm^{-2} h^{-1} in the opposite direction, which represents a larger net flux of 3.16 μmol cm^{-2} h^{-1} across the omasum to blood than observed with rumen epithelium. The mean s.c.c. was equivalent to 0.80 μmol cm^{-2} h^{-1}, representing 25 % of the Na current. So far we have only made observations of K flux in one paired experiment maintained in short-circuit throughout and this indicated a net flux of only 0.13 μmol cm^{-2} h^{-1} from blood to omasum, which is smaller than observed with rumen epithelium and, if confirmed, would only account for 4 % of the sodium current (mean current for this experiment 0.68 μmol cm^{-2} h^{-1} or 21 % of Na current). From the observations in chloride media and in terms of electrical neutrality, only 56 % and 27 % of the sodium current across rumen and omasum epithelium, respectively, could be accounted for by the mean short-circuit current and the opposed flux of potassium. In sulphate media, 79 % of the Na current across rumen epithelium could be accounted for in this way.

Anion fluxes

Sperber & Hydén (1952) first suggested that active transport of chloride might occur from rumen to blood, but Dobson & Phillipson (1958) believed that the absorption of chloride in their experiments occurred passively down the electrochemical gradient produced by the measured potential difference between rumen contents and blood (about 30 to 40 mV). Stevens (1964) found a net flux of chloride of 3.5 μmol cm^{-2} h^{-1} from rumen to blood using short-circuited preparations of rumen epithelium from cattle and goats. In our experiments, measurements of chloride fluxes using ^{36}Cl showed that even larger fluxes than seen for sodium were occurring in both directions across the epithelium (mean 3rd hour data: 4.88 μmol

$cm^{-2}\,h^{-1}$ from rumen to blood and 3.70 μmol $cm^{-2}\,h^{-1}$ from blood to rumen; three paired experiments) and there was a net active transport of chloride from rumen to blood of 1.18 μmol $cm^{-2}\,h^{-1}$ with a corresponding s.c.c. of 0.35 μmol $cm^{-2}\,h^{-1}$ (Harrison *et al.* 1968; 1970). Taking an overall mean value for the short-circuit current in the Na, K and Cl experiments $\frac{1}{3}(0.46+0.58+0.35) = 0.46$ we find that electrical balance ($1.57_{Na} - 0.46_{s.c.c.} - 0.36_{K} - 1.18_{Cl} = -0.43$) still requires additional cation transfer from rumen to blood or anion transfer from blood to rumen or both.

In three paired experiments with omasum epithelium there was a net flux of chloride of 2.01 μmol $cm^{-2}\,h^{-1}$ from omasum to blood (mean 2nd hour data: 8.94 μmol $cm^{-2}\,h^{-1}$ omasum to blood; 6.93 μmol $cm^{-2}\,h^{-1}$ blood to omasum; mean s.c.c. 0.93 μmol $cm^{-2}\,h^{-1}$). Again taking an overall mean s.c.c. $\frac{1}{3}(0.80+0.68+0.93) = 0.80$, electrical balance ($3.16_{Na} - 0.80_{s.c.c.} - 0.13_{K} - 2.01_{Cl} = +0.22$) for these omasum experiments would require a small net movement of anion from omasum to blood, or cation from blood to omasum. The small net movement of magnesium (using ^{28}Mg) recently observed across short-circuited omasum preparations would further upset the balance. However, in two other paired preparations of omasum epithelium, the net flux of chloride was apparently from blood to omasum and recalls the earlier observations of Dobson (1959) in his studies of rumen transport in anaesthetized sheep when he observed in some experiments that chloride moved from blood to rumen. Recently, Scott (1970) has presented evidence for the active transport of chloride from rumen to blood in conscious sheep.

Ionic coupling and the effects of inhibitors

Since the mean net flux of Na across rumen epithelium in experiments with sulphate media was approximately 31 % less than that in the presence of chloride anion, it appeared that part of the Na flux could be coupled to chloride ion flux. This has been confirmed in experiments in which Na fluxes were measured successively in chloride and then sulphate Ringer (or vice versa) in the same preparation. In three paired experiments, the fluxes of Na in both directions were reduced in sulphate media and the net flux was reduced by 26, 29 and 40 % respectively on replacing chloride ions by sulphate. The short-circuit current measurements were correspondingly increased by 16, 69 and 46 % in sulphate media (cf. mean increase in earlier experiments of 22 % in sulphate against chloride media).

In other experiments, treatment of both sides of the epithelium with 10 mmol/l acetazolamide considerably reduced the fluxes of chloride in both directions and reduced the net flux from rumen to blood. So far, however, we have not found clear evidence of any effect of acetazolamide on the fluxes of Na across rumen epithelium.

As previously mentioned, treatment of the blood side of the epithelium with ouabain (10^{-4} mol/l) abolishes the potential and short-circuit current. It has also been found to abolish the net flux of Na and markedly reduce that of chloride and potassium.

Scott (1967), working on conscious sheep, observed an apparent increase in the absorption of sodium from the rumen when additional K was given into the rumen. In a recent study of the effect of increasing the rumen $[K^+]$ on the fluxes of ^{22}Na across isolated rumen epithelium, there was a 50 % increase in the net flux of Na from rumen to blood in two paired experiments in open-circuit when the rumen $[K^+]$ was increased ninefold. However, in two similar experiments conducted in short-circuit to eliminate the increased electrical potential produced by

the elevated rumen [K^+], there was only a slight change in the net flux of Na when the rumen [K^+] was increased. These observations support Scott's findings and imply a coupling between Na and K transport.

Summary

Keynes (1969) has reviewed the basic types of ion pump in epithelial tissues and it would seem that rumen and omasum epithelium possess a cellular sodium pump (type I), part of which could be coupled to chloride movements in the same direction and part to potassium movements in the opposite direction. However, the fluxes of chloride in both directions across rumen and omasum are larger than those of sodium and it is likely that there is a separate chloride pump (type III). This pump may be affected by acetazolamide and perhaps works in both directions. It could account for the small negative current flux observed in most experiments after treatment of this tissue with ouabain, a known inhibitor of the sodium pump.

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