auf Agar mit Zusatz von Homogenisat aus gesunden Nadeln unterschiedlich resistenter Kiefern kultiviert und die pH-Verschiebung gemessen. Es waren pH-Erhöhungen zwischen 0,5 und 1 pH-Einheit zu verzeichnen. Zwischen den pH-Veränderungen im Nadelagar aus resistenten und anfälligen Kiefern-Klonen ergaben sich signifikante Unterschiede (p 5%). Somit besteht selbst im nicht mehr vitalen Homogenisat der Wirtspflanze ein unterschiedlicher Einfluss auf die pH-Regulation durch den Parasiten.

Das Regulationsverhalten der Wirtspflanze liess sich während der Pathogenese in vivo nicht prüfen. Bestimmend für den Widerstand, der der pH-Regulation des Parasiten entgegenwirkt, ist jedoch die Pufferkapazität des Gewebes, in dem er wächst. Diese wurde in vitro durch Titration von Nadelhomogenisat bestimmt. Dabei ergab sich für resistente Kiefern-Klone eine signifikant (p 1%) höhere Pufferkapazität als für anfällige (Figur).

Die Korrelation zwischen der für die einzelnen Kiefern-Klone ermittelten Pufferkapazität und der Verschiebung des pH-Wertes durch den Pilz in Nadelagar ergibt sich zu $r^* = -0{,}86$ (p 1%), zwischen Resistenz und Pufferkapazität zu $r^* = 0{,}84$ (p 1%). Erste Ergebnisse aus Untersuchungen an weiteren Wirt-Parasit-Systemen deuten an, dass sich ähnliche Abhängigkeiten auch dort nachweisen lassen. Dies deutet auf die Möglichkeit hin, das Merkmal Pufferkapazität in Züchtungsvorhaben zur indirekten Diagnose der Resistenz zu nutzen und aufwendige künstliche Infektionsversuche zu umgehen.

Summary. The connection between the pH-regulation of a plant (*P. sylvestris*) and the resistance to fungal parasites was studied. The host-parasite-interaction between pine and needle cast fungus showed a correlation between the buffer capacity of homogenized needles and the resistance to the pathogen *Lophodermium pinastri*.

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The Occurrence of Bacteriophages in the Rumen and their Influence on Rumen Bacterial Populations

The capacity of ruminants and other herbivores to utilize roughage containing high levels of cellulose as a source of nutrients depends on the presence and metabolic activities of symbiotic micro-organisms concentrated in anatomically specialized compartments.

In the rumen, for instance, bacteria and protozoa digest food material ingested by the host animal and use it for growth and other energy-requiring processes; there is a concomitant production of volatile fatty acids and release of CO_2 and methane. The bulk of the volatile fatty acids are absorbed by the ruminant through the rumen epithelium; digestion of the microbial cell products commences in the abomasum[1]. Any factor which affects the balance of rumen microbial populations and their rate of metabolism of the ingested food may have important effects on the nutrition of the host animal.

The total population density of bacteria in the rumen may be as high as 2.45×10^{10} ml^{-1} in a normal sheep[2], and 4.83×10^{10} ml^{-1} in the absence of ciliate protozoa[3]. Large numbers of bacteria which are not rumen inhabitants may be ingested with the feed[4], and certain aerobes such as *Lampropedia merismopedioides*, may actually grow in the presence of oxygen in the gas phase above the rumen digesta[1]. Other aerobic organisms which require a high carbon dioxide atmosphere and which have been isolated from the rumen[5] may be normal rumen inhabitants, though numerically insignificant[1].

In view of the high bacterial population density normally present, it would not be surprizing to find bacteriophages in the rumen, and indeed a variety of these are present[6–8]. Bacteriophages have been reported associated with the facultative anaerobe *Streptococcus bovis* which occurs in the rumen and a variety of other habitats[6]. Some bacteriophages have also been observed in samples of rumen liquor attached to bacterial cell walls[7], but it has not been established whether these bacteria are true rumen inhabitants or infected aerobic organisms adventitiously acquired with the feed. True rumen bacteria occur usually at high population densities, are anaerobic, will live at a temperature of 39–40 °C at a pH of 5.5–7.0 in the presence of fermentation products, at the expense of the ingesta provided by the ruminant, and may require a constituent of rumen fluid for growth[1]. A number of true rumen bacteria have been isolated and cultured in vitro, and at least two (a gram negative bacterium designated W461[9] and EADIE's Oval[10,11]) under study here have been found to be infected with bacteriophages.

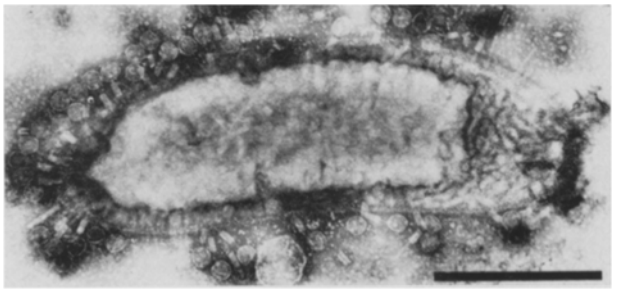

Fig. 1. Electron micrograph of W461 coated with bacteriophages (W461ψ1), negatively stained. Scale bar represents 0.5 μm.

[1] R.E. HUNGATE, *The Rumen and its Microbes* (Academic Press, New York 1966).
[2] A.C.I. WARNER, J. gen. Microbiol. *28*, 129 (1962).
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[5] R. STELLMACH-HELLWIG, Arch. Mikrobiol. *38*, 40 (1961).
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[8] N.J. HOOGENRAAD, and F.J.R. HIRD, Aust. J. biol. Sci. *23*, 793 (1970).
[9] R.W. WHITE and P. KEMP, J. gen. Microbiol. *68*, 6 (1971).
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Bacterium W461 is a gram negative curved rod which carries out biohydrogenation reactions in vitro. It was present in the rumen from which it was isolated at a population density of 10^8 ml^{-1}, and its cultural characteristics satisfy the requirements of an authentic rumen bacterium[9]; the associated bacteriophage has been designated W461ψ1.

Bacteriophage W461ψ1 has a type A structure[12], possessing a contractile tail sheath and an apparently isooctahedral head. It has been observed adsorbed in quantities of up to 50 particles per cell to a small proportion of W461 cells in liquid culture in vitro (Figure 1). Bacteriophage W461ψ1 has been carried through many subcultures of W461 picked from single isolated colonies over a period of at least 2 years. This suggests that a prophage is present in the entire population since each colony originated from a single cell. This bacteriophage is therefore temperate, and as such would not be expected to dramatically affect the population density of W461 in vivo unless the population was exposed to an inducer. Attempts to induce lysis in cultures in vitro by exposure to UV-light, hydrogen peroxide and mitomycin C have met with no success.

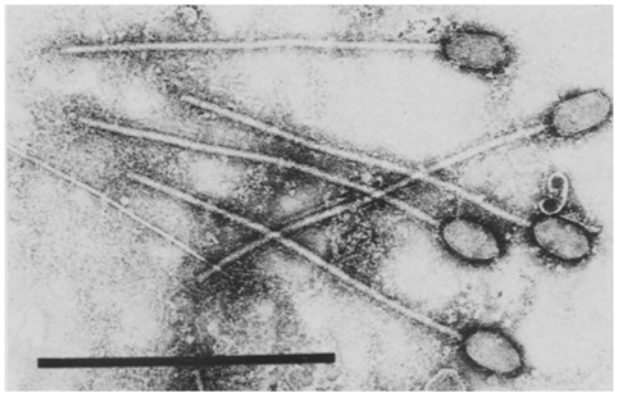

Fig. 2. Electron micrograph of bacteriophages E2ψ1, negatively stained. Scale bar represents 0.5 μm.

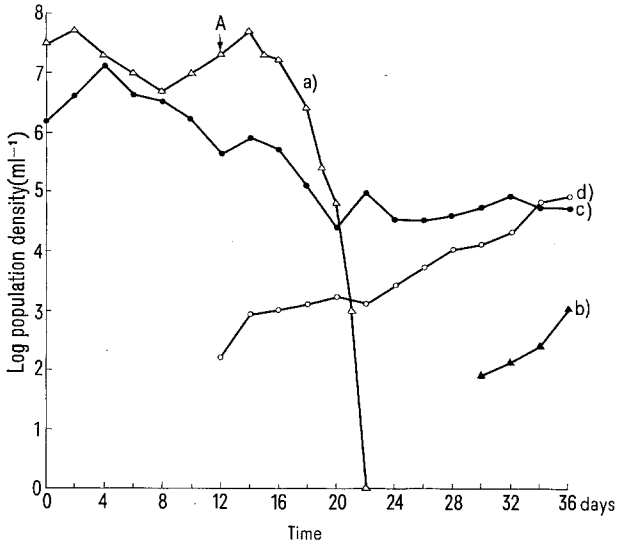

Fig. 3. Population density of EO2 in the rumen of a sheep, in the presence and absence of E2ψ1. The EO2 were originally cultured in vitro and were inoculated into the rumen of the sheep after it had been treated to remove ciliate protozoa and large bacteria. The number of EO2 (curve c) and other large bacteria (curve d) in a typical experiment in the absence of E2ψ1, was determined beginning (at T = 0) 58 days after inoculation. The entire large bacteria population (including EO2) was then removed, and the rumen reinoculated with EO2. The number of EO2 (curve a) and other large bacteria (combined) (curve b) was determined beginning (at T = 0) 30 days after the inoculation with EO2. E2ψ1 was first recorded at point A.

Eadie's Oval, which is a relatively large bacterium (12.2 μm × 8.5 μm average) occurs in the rumens of sheep at the authors' laboratory at population densities of up to 1.1×10^8 ml^{-1}, though in the majority of cases the population density is between 10^6 — 10^7 ml^{-1} [11]. 2 strains (designated EO1 and EO2) have been identified[11,13]. A bacteriophage (E2ψ1) has been found associated with EO2. Like the bacteriophage of W461, E2ψ1 also has a type A structure, but it is unusual in the great length of the tail (about 6,500 Å) (Figure 2) and in the form of the tail fibres. It was first observed by electron microscopy in negatively stained preparations of EO2 grown in vivo[14]. (For in vivo culture, EO2 are first grown in vitro and then inoculated and grown as the sole large bacterium in the rumen of sheep which have been treated to remove ciliate protonzoa and large bacteria). These preparations had been repeatedly centrifuged (250 *g*, 5 min) to free the EO2 of other bacteria and would not have been expected to contain free bacteriophages unless they derived from the EO2 themselves. Examination of thin sections of the same preparation revealed the bacteriophage particles within intact EO2 cells. The free E2ψ1 particles presumably were released from infected cells which lysed during the centrifugation or subsequent resuspension in salts solution. The EO2 normally survive this treatment without lysing, but in these preparations many of the cells were lysed.

The effect of E2ψ1 provided evidence that bacteriophages can exert a significant influence upon the population density of the host bacterium in the rumen (Figure 3). Shortly after E2ψ1 was found to be present the population density of EO2 in vivo fell from 5.2×10^7 ml^{-1} to zero, within 10 days (curve a, Figure 3). The population density of uninfected cells as shown in previous epxeriments using EO2, the same sheep, fed the same diet, was maintained at a high level for several weeks (curve c, Figure 3) until other, apparently competing, large bacteria (Quin's Ovals, large Selenomonads and *Oscillospira guilliermondii*) became established (curve d, Figure 3). The decrease in population density of the infected EO2 (curve a, Figure 3) was not coincident with a rise in the population densities of the other large bacteria (curve b, Figure 3). The EO2 were not detected for a further 36 days after which small numbers were again observed and the EO2 population density then fluctuated between 2×10^5 ml^{-1} and zero during the next few months. 3 subsequent attempts to boost the population by the addition of 10^9 EO2 grown in vitro[11] and free of bacteriophage particles, failed. This number of EO2 would normally have been sufficient to establish the EO2 at a high population density within 2–4 weeks.

Infected EO2 cells were also added to the rumen of a second sheep containing 8.2×10^6 ml^{-1} of normal uninfected cells; this resulted in a dramatic fall in the population density to zero after 7 days; E2ψ1 was present during the decline. There were no EO2 present during the next 3 months.

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[13] C.G. Orpin and E.A. Munn, unpublished observations.
[14] C.G. Orpin, J. gen. Microbiol. *68*, 17 (1971).

There is no evidence to suggest that E2ψ1 was present in the EO2 as a temperate phage; had it been temperate and become induced in vivo due to a component in the sheep's diet, then, assuming a uniform distribution of the inducer in the rumen, all the cells in the population would lyse within a short time. If, due to uneven mixing within the rumen, only a proportion of the EO2 were exposed to the inducer, there would only be a partial drop in the population density. Complete and gradual lysis could only be explained by postulating a regular intake of inducer which did not mix uniformly in the rumen; this is unlikely, however, since the same diet had been fed for more than 1 year during periods of high EO2 population densities and had been fed to other sheep containing EO2 in which bacteriophages did not subsequently appear. Bacteriophage E2ψ1 is therefore probably a virulent and not an induced temperate bacteriophage.

Recognition of the presence of both temperate and virulent bacteriophages in the rumen therefore brings another factor for consideration into the already complex field of ruminant nutrition and rumen ecology. Although it is as yet not known if other rumen bacteria have bacteriophage pathogens the number and variety of bacteriophage particles present in the rumen suggest that this is so. Temperate bacteriophages would not be expected to have a dramatic effect on the population densities of individual bacterial species unless that population was subjected to induction, perhaps by a plant constitutent in the diet. Virulent bacteriophages, on the other hand, as shown by the effect of E2ψ1 on EO2 would have a radical effect on specific populations. In terms of the well-being of the host animal, lysis of bacteria in the rumen with secondary fermentation of the products would involve losses of carbon compounds in the form of gases, but this would be partially offset by increased volatile fatty acid production. The effect of the loss of a single species, even of the important cellulolytic bacteria would probably be temporary because other species capable of occupying the same ecological niche would proliferate. Since, however, inducers of temperate phages are non-specific in their action, should such an inducer occur in the diet it is possible that several species could be lost simultaneously.

Resumen. Se han demostrado que dos cultivos de bacterias de rumen (un bastón gram negativo, designado W461, y una cepa de óvalos de EADIE) están infectados con bacteriófagos. El bacteriófago de W461 es templado; aquel de los óvalos es virulento y depleta rápidamente la población bacteriana en vivo.

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Pattern of Electrolyte Leakage in Downy Mildew Affected Sorghum

Alteration of permeability and ionic imbalance associated with plant disease development has been reported by many workers[1]. The present investigation reveals the pattern of electrolyte leakage in sorghum plants systemically affected by downy mildew, incited by *Sclerospora sorghi*.

Materials and methods. A highly susceptible variety (DMS 652) was used for the present investigation. Systemic infection was obtained following a previous method[2].

The loss of electrolyte was estimated by measuring the conductivity of the leachates. The electrolyte leakage was assessed from the roots, stem and leaves after 15, 30 and 45 days of sowing. The plant parts were initially washed thoroughly with tap water, subsequently with several changes of distilled water and finally rinsed with double distilled water. They were blotted dry and were suspended in sterile double distilled water in the ratio of 1 g of plant part to 10 ml of double distilled water. The conductivity of the bathing solution was measured after incubation at 25 °C for 24 h. All conductivity measurements were made with an ELICO model CM-82 conductivity bridge with platinum blackened electrodes. The temperature of the bathing solution was maintained at 25 °C during conductivity measurements. The results are expressed as specific conductivity (μmh/cm) of the leachates.

Results and discussion. The various parts of sorghum plant exhibited a pattern of loss of electrolytes and permeability change as a result of downy mildew attack (Table).

The greater leakage of electrolytes from diseased roots and leaves may be due to the higher absorption of electrolytes by diseased plants from the soil. Being an obligate

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Specific conductivity of bathing solutions of different parts of sorghum attacked by *Sclerospora sorghi*

Days after planting	Specific conductivity (μm h/cm) of bathing solutions of					
	Roots		Stems		Leaves	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
15	252.4	430.4	259.4	184.2	433.2	480.0
30	256.2	427.2	270.9	186.9	453.5	691.9
45	263.6	447.3	296.0	197.3	890.0	1530.8