number of mutant forms (nucleotide sequences) that are equally functional and fit in adaptation. All these mutant forms will be a source of neutral evolution.

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Note added in proof: After submission of this paper, we learned that Miyata and Yasunaga[22] estimated the rate of nucleotide substitution for mouse $\psi\alpha 3$. Their estimate is higher than ours, because they did not exclude the middle part of the sequence.

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Gait and the energetics of locomotion in horses

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It seems reasonable that quadrupeds should change gait from a walk to a trot to a gallop in such a way as to minimize their energy consumption, as human beings are known[1] to change from a walk to a run at a particular speed (2.4 m s^{-1}) below which walking requires less energy than running and above which the opposite is true. Thus by changing gait, human beings keep the energy cost of locomotion to a minimum as their speed increases. One reason this relation holds is that in humans, metabolic rate increases curvilinearly with walking speed[1]. If metabolism were a curvilinear function of speed within each of the gaits used by quadrupeds, it would support the hypothesis that they also change gait to minimize energetic cost. There is an old controversy about whether metabolic rate increases linearly or curvilinearly in running humans[1,2] but all previous reports have suggested that metabolic rate increases linearly with speed in quadrupeds. Extended gaits were an important experimental tool in the study of human gait changes; thus we have trained three small horses (110–170 kg) to walk, trot and gallop on a motorized treadmill, and to extend their gaits on command. We report here that, using measurements of rates of oxygen consumption as an indicator of rates of energy consumption, we have confirmed that the natural gait at any speed indeed entails the smallest possible energy expenditure.

Rate of oxygen consumption increased curvilinearly with speed for walking and trotting (Fig. 1). We were unable to obtain sufficiently high galloping speeds to evaluate whether rate of oxygen consumption also increased curvilinearly during a gallop. Transitions between gaits normally occurred at the speeds where the curves intersected and oxygen consumption was the same for the two gaits. When the gaits were extended beyond their normal range of speeds, oxygen consumption was higher in the extended gait than in that which the animal would normally be using. For example, horse B normally walked at a speed of 1.25 m s^{-1}. When trotting at 1.25 m s^{-1}, the rate of oxygen consumption was 1.5 times that measured during a walk.

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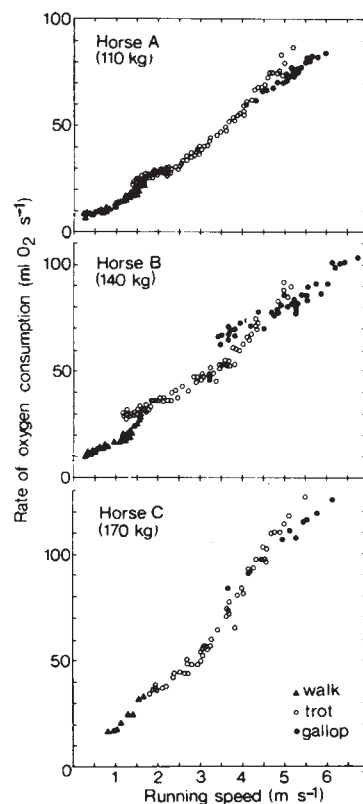
Fig. 1 Steady-state rate of oxygen consumption as a function of speed for a walk, a trot and a gallop in three small horses. The horses normally changed from walk to trot, or trot to gallop at the point where rate of oxygen consumption was the same in the two gaits. We were able to train the animals to extend their gaits to speeds where they would have normally used a different gait. The data from the extended gaits accentuated the curvilinear nature of the relationship between rate of oxygen consumption and speed within a gait. Linear regression provided a good fit of the data if we omitted measurements made when animals used extended gaits. The least-squares regressions and coefficients of determination (r^2) are: horse A, $\dot{V}_{O_2} = -1.22 + 14.4 \cdot v$, $r^2 = 0.99$; B, $\dot{V}_{O_2} = 2.68 + 15.0 \cdot v$, $r^2 = 0.98$; C, $\dot{V}_{O_2} = -8.61 + 22.2 \cdot v$, $r^2 = 0.98$ where $\dot{V}_{O_2}$ is ml O$_2$ s^{-1} and v is velocity in m s^{-1}. We considered that a steady-state oxygen consumption was achieved when we recorded a constant rate of oxygen consumption for at least 5 min. At the speeds used here, steady-state was never reached during the first 6 min of measurement, thus all measurements at a given speed were made for at least 11 min. During these measurements, air temperature averaged 21 °C and air speed was approximately matched to tread speed. The animals wore loose-fitting masks which allowed capture of all expired gases. Rates of gas flow through the mask were varied from 150 l min^{-1} to 1,300 l min^{-1} during different runs to produce excurrent oxygen concentrations between 20.6 and 20.1%. During the runs the laboratory was ventilated by a large fan and ambient oxygen concentrations remained constant at ~20.9%. Previous studies of horses, including pony B of ref. 4, found no change in blood lactate levels when animals ran on a level treadmill at speeds up to 10 m s^{-1}; therefore we conclude that there is no anaerobic contribution to energy consumption at speeds <7 m s^{-1}.

There was a speed for each gait where the amount of oxygen used to move a given distance (rate of oxygen consumption divided by speed) reached a minimum value (Fig. 2). This speed represents one that is energetically optimal for each gait. The minimum values were about the same for a walk, a trot and a gallop. Therefore, the amount of energy consumed by a horse to move a given distance is the same at these optimal speeds.

To determine whether animals normally moved at energetically optimal speeds, we measured speeds and gaits of a horse as it moved freely over a marked grid. We found that the horse selected speeds within each gait around the energetically optimal speed (Fig. 2). Pennycuick[3] has observed that migrating African animals use only a restricted range of speeds within each gait. This suggests that these animals might also be using an energetically optimal speed for each gait.

Linear regression of the data provides a reasonable fit to the relationship between oxygen consumption and speed if one disregards the data obtained when the animals had abnormally extended gaits ($r^2 = 0.98$). In fact the curvilinear relationship
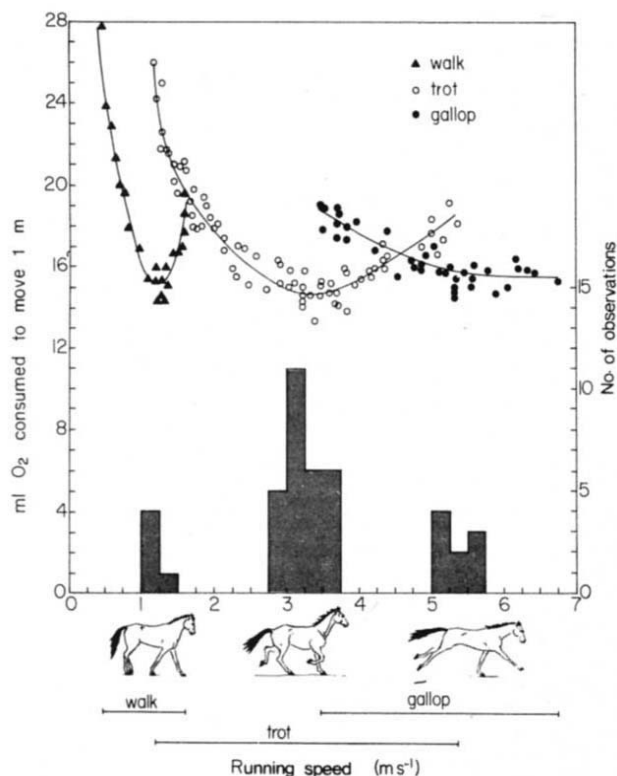
can only be observed when great care is taken to avoid any source of variability in the data. Therefore, it seems useful to continue to use a single linear relationship between rate of oxygen consumption and speed for comparative studies of energetics of locomotion.

We conclude that horses, like humans, change gait and select speed within a gait in a manner that minimizes energy consumption. Observations of speeds used by migrating African animals[3] suggest that this finding may also apply generally to terrestrial animals.

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Gene expression in visna virus infection in sheep

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Visna is a slow degenerative disease of the central nervous system (CNS) of sheep caused by a nontumorigenic retrovirus[1]. During the course of this disease, visna virus establishes a persistent infection of the CNS, lung and haematopoietic system, despite a specific humoral and cellular immune response. We have studied visna virus life cycle at the single-cell level in choroid plexus of experimentally infected animals, using a very sensitive and quantitative *in situ* hybridization assay[2]. We report here that although proviral DNA is synthesized in significant amounts, its expression is blocked at the transcriptional level. This restriction of proviral DNA transcription offers an explanation for the slowness of the disease and the persistence of the infection.

Two aspects of visna infection are relevant to the question of slowness and persistence. First, the virus is predominantly cell-associated, especially in the blood; second, the amount of virus present at any time during the course of the disease is minimal[3]. This limitation in virus growth is not a consequence of the immune response because it is observed before virus-specific antibodies can be detected, and because immunosuppression of infected animals does not lead to a higher titre of virus[4]. Restricted virus replication therefore seems to be an intrinsic property of the virus–cell interaction in the tissues of the animal (*in vivo*). This *in vivo* situation is in sharp contrast to virus replication in ovine cells grown *in vitro*, where the virus grows to high titre and causes a characteristic cytopathic effect that culminates in cell death 48–72 h after infection.

The first clue to the mechanism of restriction of replication *in vivo* was revealed by studies which showed that proviral DNA can be detected by *in situ* hybridization in a significant fraction

of the cells in the choroid plexus, but that only an occasional cell contains the major structural viral polypeptide p30 as detected by immunofluorescence[5]. This restriction of proviral DNA expression provided an explanation for the persistence of the infection: the immune defence mechanisms are unable to eradicate the infection because the virus persists intracellularly in cells that do not express viral antigens. Therefore, an understanding of the mechanism of restriction of viral gene expression is central to the study of visna pathogenesis.

We chose to study viral replication in the choroid plexus because histopathological lesions of visna always predominate in this tissue as well as in the periventricular areas of the brain[3], and because persistent infection of the choroid plexus has been clearly documented by isolation studies[3]. Although it is a relatively simple structure, the choroid plexus consists of various cell types (cuboidal epithelium cells, fibroblasts, endothelial cells and smooth muscle cells) interspersed with large numbers of inflammatory cells characteristic of visna lesions. Because of this complex setting and the possibility of local variations in the extent of infection, virus replication must be studied at the single-cell level. Viral protein synthesis was assessed by immunofluorescence using an indirect assay and the labelled avidin–biotin method of detection[6]. Virus nucleic acids were quantitated in single cells in histological sections by *in situ* hybridization[2] modified for the detection of proviral DNA as described in Fig. 1 legend. This assay will detect 1 to 2 copies of viral RNA and 10 copies of proviral DNA per cell after three weeks of autoradiographic exposure (10 grains per cell above background).

Three American lambs were inoculated intracranially with 10^9 plaque-forming units (PFU) of visna virus strain 1514. The inoculum (1 ml) was deposited in the vicinity of the lateral ventricule. A large inoculum was chosen to infect a maximum number of cells at the site of inoculation. The animals were killed between 7 and 15 days after inoculation, and the choroid plexi removed. Inflammatory lesions typical of visna were observed in all cases. A portion of choroid plexus was frozen, subsequently sectioned in a cryomicrotome and assayed for viral nucleic acids and proteins. Examples of the results obtained by *in situ* hybridization are shown in Fig. 2. Proviral DNA and viral RNA were readily detected in these sections. In all cases where