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First, various colonies from ruminal fluid were formed on Müller-Hinton agar plates or Soybean-casein-digest agar plates containing 5% sheep blood after incubation at 37°C for 1~2 days under aerobic or anaerobic conditions. Each colony formed on the plates was inoculated to a sterile medium consisting of 2 ml of ruminal fluid from sheep and 500 µl of onion juice, and incubated at 38.5°C for 48 hours under anaerobic conditions. After incubation, 2 ml of the supernatant of the medium was partitioned with diethylether. After the ether was removed, the remaining dry materials were added to a sheep erythrocyte suspension. The suspension was incubated at 38.5°C for 2 hours, and the methemoglobin concentration of sheep erythrocytes was measured. The colonies that formed a high methemoglobin concentration (more than 60%) were selected as "active bacteria" strains.

Consequently, 5 strains from 2 sheep, 11 strains from 2 cows, and 1 strain from 5 goats were isolated as active bacteria. One strain of active bacteria, derived from sheep, was identified as *Lactococcus lactis lactis*, and the others were identified as *Streptococcus bovis*.

Antimicrobial sensitivity tests were performed for the *Streptococcus bovis* isolated from sheep. These tests showed that *Streptococcus bovis* was susceptible to penicillin G, ampicillin, dicloxacillin, cefalexin, tetracycline, erythromycin, lincomycin and chloramphenicol.

From the results, it was demonstrated that *Streptococcus bovis* was mainly responsible for the onset of onion-induced hemolytic anemia in ruminants. In addition, the antibiotics described above may be useful for the treatment of the disease.

#### Identification of a genetic defect and the establishment of gene diagnosis of cutaneous asthenia in a Holstein cow

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A defect of the core protein of dermatan sulphate proteoglycan was suspected to account for the pathogenicity of a Holstein cow affected with cutaneous asthenia (P1). By genetic analysis of the core protein in P1, a point mutation was detected. The mutation was a G to A transition at nucleotide position 254, which resulted in a Ser to Asn substitution of the bovine proteoglycan core protein. This substitution occurred in one of the Ser-Gly dipeptide repeats, which was considered the binding portion of dermatan sulphate. The dermatan sulphate chain can bind to Ser residues by an O-glycosyl bond, but can not

bind to an Asn residue because of the different pattern of the glycosyl bond. Cutaneous asthenia in P1 is, therefore, a disorder with mutation of the gene for the core protein of dermatan sulphate proteoglycan.

An attempt at genetic diagnosis of this type of cutaneous asthenia was then made using restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR). The results of RFLP and PCR indicated that P1 was a heterozygote of an abnormal gene of core protein. Thus, it was strongly suggested that the cutaneous asthenia in P1 was an autosomal

dominant disorder.

These data suggested that the functional abnormality of cutaneous tissues of P1 was due to

an abnormal gene of core protein that induced amino acid substitution.

### Studies on the cause of increased multiplication of *Babesia gibsoni* in reticulocytes

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It has been reported that *Babesia gibsoni* shows greater multiplication when it is cultured together with canine reticulocytes, immature red blood cells (RBCs), as compared with erythrocytes, mature RBCs. The purpose of this study was to clarify the factors which enhance the multiplication of *B. gibsoni* in canine reticulocytes *in vitro*. First, *B. gibsoni* parasites were cultured together with the following four different kinds of resealed RBCs, resealed mature RBCs (M[M]RBCs), resealed reticulocytes (R[R]RBCs), resealed mature RBCs containing reticulocyte hemolysate (M[R]RBCs), and resealed reticulocytes containing mature RBC hemolysate (R[M]RBCs). As a result, a significant ( $p < 0.05$ ) increase of the parasites was observed in the culture with either R[R]RBCs or M[R]RBCs, compared to the culture with M[M]RBCs. These results suggested that the factor which enhances the multiplication of the parasites cultured in reticulocytes was present within the hemolysate of reticulocytes.

Second, the hemolysate of reticulocytes was

filtrated using a 0.45  $\mu\text{m}$  pore filter, or centrifuged at  $20,000 \times g$  for 20 minutes. Resealed reticulocytes containing the filtrate (R[FR]RBCs) or the supernatant of the centrifuged hemolysate (R[CR]RBCs) were prepared. Resealed mature RBCs containing sediments of the hemolysate were also prepared (M[PR]RBCs). When the parasites were cultured together with R[FR]RBCs or R[CR]RBCs, multiplication of parasites was significantly ( $p < 0.05$ ) lower compared to the culture with R[R]RBCs and similar to that with M[M]RBCs. Furthermore, the multiplication of the parasites cultured with the M[PR]RBCs was significantly ( $p < 0.05$ ) increased compared to that with M[M]RBCs, and reached the same level as that with R[R]RBCs. Electron microscopic observation revealed that M[PR]RBCs have many vacuoles, containing small granules, and a few mitochondria.

These results indicate that an intracellular organelle, such as mitochondria, may be an important factor which enhances the multiplication of *B. gibsoni* in canine reticulocytes.