PrP 3F4, Sigma-Aldrich, Steinheim, Germany, dilution: 1:4,000 in PBS and 1.5% BSA or monoclonal anti-mouse 6H4, Prionics AG, Zürich, Switzerland, 1:3,000 in PBS). After washing 5 times with PBS for 5 min and 0.05% Tween 20 (Perbio Science GmbH, Heidelberg, Germany), blots were incubated in a secondary antibody solution (horseradish peroxidase-conjugated anti-mouse, dilution 1:500,000 Perbio Science GmbH or alkaline-phosphatase-conjugated anti-mouse, dilution 1:5,000 Sigma-Aldrich, Steinheim, Germany) for at least 1.5 h. Membranes were washed as described before and developed with CDP-Star solution (Invitrogen GmbH, Karlsruhe, Germany) or SuperSignal West Femto Maximum Sensitivity substrate (Perbio Science GmbH, Heidelberg, Germany) for 5 to 10 min. Protein signals were visualized using Pierce film (Perbio Science GmbH, Heidelberg, Germany).

For confirmation of these results, highly sensitive Western blotting was performed in independent experiments at different laboratories using their established protocols.

Western Protocol II

The protein extractions from contaminated soil samples were carried out by adding 2 ml 1%-SDS-solution to 2 g of testing material in a 15 ml tube. The suspension was vigorously shaken for 2 min, followed by a centrifugation step at 7,000 rpm for 10 min. 50 µl of the clear supernatant was used for proteinase K digestion (100 µg/ml; 37°C; 1 h). After digestion the supernatants were mixed with an equal volume of 2× sample loading buffer (50 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 50 mM ß-mercaptoethanol and 0.001% bromphenol blue) and heated to 100°C for 5 min for PrP-Western blotting.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyzes of samples from hamsters were performed as described elsewhere [58] with recently published modifications [39]. In short: Following SDS-PAGE, proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilon, Millipore, Billerica, USA) using a semi-dry blotting system. The membrane was blocked by incubation for 30 min in TBS containing 3% (w/v) nonfat milk powder (NFMP) and 0.05% (w/v) Tween-20 (NFMP-TBST). Blots were incubated overnight in primary antibody solution (monoclonal anti-PrP antibody 3F4 [mAb 3F4] from cell culture, diluted 1:2,000 in NFMP-TBST) at 4°C. After washing 5 times for at least 20 min with NFMP-TBST, blots were incubated in secondary antibody solution (alkaline phosphatase conjugated goat anti-mouse IgG; Dako, Glostrup, Denmark; 1:5,000 diluted in NFMP-TBST) for 90 min at room temperature. After washing 5 times with NFMP-TBST over a total period of at least 1.5 h, the membranes were pre-incubated 2×5 min in assay-buffer (100 mM Tris, 100 mM NaCl, pH 9.5) and finally developed with CDP-star solution (Tropics, Applied Biosystems, Beford, USA) for 5 min according to the instructions of the manufacturer. PrP-signals were visualized on a X-OMAT AR (Kodak, Sigma-Aldrich, Steinheim, Germany) film. Films were exposed for 5–30 min.

For deglycosylation, 500 µl of the soil extract solution were dialyzed in PBS for 16 h in order to remove the detergents, and 20 µl of the aliquot was digested using PNGase F (New England Biolabs, Ipswich, USA) according to the instructions of the manufacturer prior to Western blotting.

Western Protocol III

Sample preparation and extraction of the prion protein from soil samples as well as proteinase K digestion was performed as described in protocol I. After electrophoresis using 16% bis-acrylamide gels, proteins were transferred on a PVDF-membrane in a semi-dry chamber. The membranes were blocked in 5% dry fat milk in PBS (phosphate buffered saline) containing 0.1% Tween 20 (Merck, Darmstadt, Germany) (PBS-T) for 30 min and subsequently incubated with the PrP-specific monoclonal detection antibody 3F4 (Chemicon International, Inc., California) in a dilution of 1:3,000 in 5% dry fat milk in PBS-T for 1 h 30 min. The membranes were washed 3 times for 10 min with PBS-T and then incubated with a secondary antibody bound to alkaline phosphatase in a dilution of 1:2,000 in PBS-T (goat-anti-mouse-AP, Dianova, Hamburg, Germany) for 1 h. After again washing three times for 10 min, the membranes were incubated 2 times for 2 min in assay buffer containing 200 mM Tris-HCl and 10 mM $MgCl_2$ (pH 9.8). Finally, the chemiluminescence substrate CDP-Star (Tropix, Bedford, USA) was applied and incubated on the membrane for 5 min before the light signals were detected in a camera using the analysis software Quantity One (Bio-Rad, Munich, Germany).

Bioassay in Syrian hamsters

Forty two female Syrian hamsters, approx. 90 days old, were obtained from Charles River Laboratories, Germany. They were handled according to the regulations of the local authorities (Bezirksregierung Arnsberg, reference number 50.8735.1 Nr. 108/1) in a biosafety Level 3 containment facility.

12 animals were fed weekly over a period of 12 weeks; 11 times with 100 mg soil/brain mixture each and one time with 50 mg soil/brain mixture taken from the outdoor lysimeters after an incubation period of 26 and 29 months (table 1). Oral application was achieved by mixing the soil (1,150 mg in total) with commercial hamster feed (ssniff, Soest, Germany).

To analyze the remaining infectivity in the aqueous extract, 10 g contaminated soil was mixed with 10 ml water and was vigorously shaken on a horizontal shaker for approx. 1 h. The mixture was centrifuged with 5,000 rpm for 5 min and the supernatant was used for the bioassay. Oral application was performed weekly over a period of 12 weeks (1,150 µl in total) by mixing the extract with commercial hamster feed (ssniff, Soest, Germany). For this purpose, 12 additional animals were fed 11 times with 100 µl and 1 time with 50 µl aqueous extract from the soil/brain mixture taken from the outdoor lysimeters after an incubation period of 26 and 29 months.

The hamsters of both groups were monitored at least twice a week for the development of clinical signs of scrapie. Hamsters diseased with 263K scrapie showed head bobbing, ataxia of gait and generalized tremor. Such animals were frequently and persistently in motion, easily irritated by noise and touch, upon which they often twitch, and had difficulties maintaining balance and rising from a supine position. These clinical symptoms of hamsters are entirely consistent with those previously reported for the 263K scrapie agent [59]. At the occurrence of first neurological symptoms, the animals were observed daily and at appropriate end-points, clinically affected hamsters were killed. Brains from these hamsters were examined and analyzed for the presence of PrP^{Sc}.

As a control, six animals were fed with non-contaminated standard soil and 6 animals were fed with aqueous extract from non-contaminated standard soil over a period of 12 weeks (1,150 mg or 1,150 µl in total). Additional 6 hamsters were fed only with commercial hamster feed (ssniff, Soest, Germany).

PMCA-Reaction

The PMCA method was carried out as reported previously [45,46] with modifications: In brief, normal hamster brains were

homogenized at 10% (w/v) in PBS containing complete protease inhibitor cocktail (Boehringer-Ingelheim, Mannheim, Germany), 4 mM EDTA and 1% Triton-X-100. The homogenate was briefly centrifuged at 2,000 g and the supernatant was used as the PrP^C source. Amplification was performed using an automatic ultrasonification apparatus (Sonicator 300 from Misonix, New York, USA) with a water-tank.

The PrP^C substrate was mixed with 1/10 volume of the soil extract previously dialyzed in PBS, resulting in a total volume of 100 µl. PMCA amplification was performed by 40 cycles of sonication (40 sec. each) followed by incubation at 37°C for 1 h in the water-tank. The amplified product of the first round of amplification was diluted 1:5 with normal brain homogenate and the second round was performed. This process was repeated 4 times to obtain 160 cycles of PMCA. From each PMCA-amplification round, aliquots of 50 µl were taken and digested with proteinase K (180 µg/ml) for 1 h at 56°C. Finally, the same volume of 2× sample buffer was added and heated for 10 min at 100°C prior to SDS-PAGE.

Western Blot Typing of PrP27-30

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyzes of samples from hamsters for characterization of the PrP^{Sc} glycosylation and migration pattern were performed as described elsewhere [59]. In short: 50 µl of 10% (w/v) brain homogenates in TBS (pH 7.4) were mixed with 5 µl of 13% (w/v) sarcosyl and 10 µl proteinase K stock solution (1 mg/ml; Roche, Mannheim, Germany) and digested for 60 min at 37°C. The digestion was stopped by adding 435 µl 2× sample buffer, i.e. 4% (w/v) SDS, 10% (v/v) 2-mercaptoethanol in 120 mM Tris-HCl, pH 6.8, containing 20% (w/v) glycerol and 0.05% (w/v) bromophenol blue, and boiling for 5 min. 5 µl of the solution (corresponding to 5×10^{-5} grams of homogenized brain tissue) were separated in a 15% SDS-PAGE or in Tris-glycine gels (Novex, Invitrogen, Carlsbad, USA) and subsequently blotted onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, USA) using the semi-dry method (Fast-Blot; Biometra, Göttingen, Germany). The PVDF-membranes were blocked with 5% low-fat milk in TBS for 30 min and incubated overnight at 4°C with MAb 3F4 (1:2,000) in 3% bovine serum albumin (BSA) in TBS. After washing in TBS and incubation for 60 min at room temperature with the secondary antibody [biotinylated goat anti-mouse IgG (1:2,000) in 3% BSA in TBS], a biotin-streptavidin kit (Dako, Glostrup, Denmark) for signal enhancement was applied for 30 min at room temperature. After washing the membranes in TBS, antibody binding to PrP was visualized using a mixture of nitroblue-tetrazoleum (NBT; Sigma-Aldrich, Steinheim, Germany) and 5-bromo-4-chloro-3-indolylphosphate (BCIP; Sigma-Aldrich, Steinheim, Germany) as substrate.

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Author Contributions

Conceived and designed the experiments: MB AT MG AB BS RP KT. Performed the experiments: AT AB BS RP. Analyzed the data: MB AT MG AB BS RP KT. Contributed reagents/materials/analysis tools: MB AT. Wrote the paper: MB AT MG AB BS KT.

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研究報告の概要

プリオン感染性及びその分子マーカーである病原性プリオン蛋白 PrP^{Sc} は、中枢神経系に蓄積するとともに、しばしばTSEに罹った動物又はヒトのリンパ組織にも蓄積する。最近、以前にはプリオンに侵入されないと考えられていた組織（例えば骨格筋）内に PrP^{Sc} が発見された。我々は、経口的又は非経口的にスクレイピーを投与したハムスターにおいて、プリオンが皮膚をターゲットにするか、臓器に PrP^{Sc} の蓄積が広い範囲にわたってみられるかという問題に取り組んだ。スクレイピーを経口的に投与したハムスターでは、PrP^{Sc} は症状発現前に検出されたが、皮膚に関係した PrP^{Sc} の大部分は、臨床症状が現れた段階で蓄積した。PrP^{Sc} は皮膚内部の神経線維に局在していたが、ケラチン生成細胞には局在しておらず、皮膚での PrP^{Sc} の蓄積は、感染の経路及びリンパ球向性の伝播に依存しないことを示した。このデータは、プリオンが神経によって仲介され、皮膚へ遠心的に拡散することを示した。さらに追跡試験によって我々は、スクレイピーに自然感染した羊を調べ、5頭の内2頭の皮膚の検体中からウエスタンブロットによって PrP^{Sc} が検出された。皮膚がプリオンの貯蔵臓器とする我々の所見について疾患の伝播と関連した調査をさらに実施する必要がある。

報告企業の意見	今後の対応
実験的感染及び自然感染によってスクレイピーに罹った動物の皮膚に病原性プリオン蛋白 PrP^{Sc} が蓄積したとの報告である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1) 略
1) 略
2) 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

313

38

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Accumulation of Pathological Prion Protein PrP^{Sc} in the Skin of Animals with Experimental and Natural Scrapie

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Prion infectivity and its molecular marker, the pathological prion protein PrP^{Sc}, accumulate in the central nervous system and often also in lymphoid tissue of animals or humans affected by transmissible spongiform encephalopathies. Recently, PrP^{Sc} was found in tissues previously considered not to be invaded by prions (e.g., skeletal muscles). Here, we address the question of whether prions target the skin and show widespread PrP^{Sc} deposition in this organ in hamsters perorally or parenterally challenged with scrapie. In hamsters fed with scrapie, PrP^{Sc} was detected before the onset of symptoms, but the bulk of skin-associated PrP^{Sc} accumulated in the clinical phase. PrP^{Sc} was localized in nerve fibres within the skin but not in keratinocytes, and the deposition of PrP^{Sc} in skin showed no dependence from the route of infection and lymphotropic dissemination. The data indicated a neurally mediated centrifugal spread of prions to the skin. Furthermore, in a follow-up study, we examined sheep naturally infected with scrapie and detected PrP^{Sc} by Western blotting in skin samples from two out of five animals. Our findings point to the skin as a potential reservoir of prions, which should be further investigated in relation to disease transmission.

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Introduction

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are fatal neurodegenerative diseases affecting both animals and humans. According to the prion hypothesis, TSEs are caused by infectious prions that consist essentially—if not entirely— of a misfolded form of the prion protein (PrP), which is known as PrP^{Sc} [1]. Although the precise molecular composition and structure of prions remains elusive, PrP^{Sc} has been shown in many studies to accumulate together with infectivity in target tissues of infection and is therefore considered a reliable biochemical marker for TSE agents [2] as reported for experimentally challenged hamsters [3], other animal species [4], and humans [5,6].

Scrapie of sheep and goats, chronic wasting disease (CWD) of deer, bovine spongiform encephalopathy (BSE) of cattle, and variant Creutzfeldt-Jakob disease (vCJD) of humans represent acquired prion diseases that are caused by exposure to TSE agents in the living environment of the respective host. Different lines of evidence suggest that many, if not the majority, of cases of ovine scrapie, BSE, and purportedly CWD are caused by ingestion of prions and subsequent invasion of the organism via the alimentary tract [7]. This also holds true for vCJD, which is now generally acknowledged to be acquired through consumption of BSE-contaminated foodstuffs [8].

Although the exact mechanism of infection following passage of prions through the alimentary tract has not yet been completely elucidated, findings from different mammalian species suggested that the infection ascended retrogradually via peripheral nerves to the spinal cord and to the brain (for reviews see [2,7]). From these sites of initial central nervous system invasion at the level of the thoracic spinal cord and the medulla oblongata, the infection propagated in both ascending and descending directions [2,3,7,9–11]. Centrifugal spread from the central nervous system appeared to be responsible for subsequent infection of further parts of the peripheral nervous system [9,11]. In particular, PrP^{Sc} was found associated with nerve fibres or nerve endings innervating peripheral organs and tissues such as muscles [11–14]. This prompted us to look for further tissues which could serve as reservoirs of prions in the mammalian body, and from which these pathogens could be potentially disseminated into the environment and transmitted to other individuals via peroral or alternative routes. In this context, the skin appears to be of utmost importance. The skin

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Abbreviations: BSE, bovine spongiform encephalopathy; BSE-H, hamster-adapted BSE; CWD, chronic wasting disease; dpi, days post infection; f.p., foot pad; H&E, haematoxylin and eosin; i.c., intracerebral; PET, paraffin-embedded tissue; p.o., oral; PrP, prion protein; PrP27–30, protease-resistant core of the pathological prion protein PrP^{Sc}; PrP^{C}, cellular isoform of the prion protein; PrP^{Sc}, pathological isoform of the prion protein; s.w., steel wires; TSE, transmissible spongiform encephalopathy; vCJD, variant Creutzfeldt-Jakob disease

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Author Summary

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are fatal neurodegenerative diseases affecting the central nervous system. According to the prion hypothesis, TSEs are caused by [illegible]

consists of different strata and appendages which are highly innervated and interspersed with lymphatics and blood vessels [15]. It constitutes the largest organ of humans and many animal species and provides an interface with their environment. However, although PrP^{Sc} detection has been reported for mucosal tissue [16,17], the skin has not been extensively studied for the presence of prions and PrP^{Sc} so far. In 2004, Cunningham et al. reported on the presence of BSE agent in a wide range of tissues from a BSE-infected greater kudu [18]. In one animal of this study, the salivary gland and skin were found to contain infectivity, and the authors suggested that these findings possibly indicate routes by which direct animal-to-animal transmission of the disease may occur.

Here, we examined the skin of prion-infected hamsters for the presence of PrP^{Sc}. Our hamster experiments focussed on orally infected animals, which have been previously established as a relevant rodent model to study the spread of prions in the peripheral nervous system [2,7]. These studies were performed in order to (i) investigate whether anatomical structures within the skin may provide a target for PrP^{Sc} accumulation, (ii) elucidate the identity of such skin components, and (iii) find out whether prions can be present in the skin prior to the onset of visible TSE symptoms. In a proof-of-concept approach, we extended PrP^{Sc} testing of the skin to specimens from sheep naturally infected with scrapie. This follow-up study intended to obtain further insights into the pathophysiology of scrapie and the putative pathways of its natural transmission in the field.

Results

PrP^{Sc} Accumulation in the Skin following Peroral Infection with Scrapie Becomes Detectable Shortly before the Onset of Clinical Symptoms

To investigate whether and at which stages of scrapie infection PrP^{Sc} accumulates in the skin, we performed a time-course study in hamsters orally exposed to 263K scrapie agent. As established previously for the examination of muscle tissue [11], PrP^{Sc} was visualized by sensitive Western blotting after extraction of the protein in the form of its protease-resistant core, PrP27–30, from skin specimens by a high-yield purification method.

We examined samples of skin tissue from hamsters taken at five different time points, i.e., at 70, 100, and 130 days post infection (dpi) in the pre-clinical phase of incubation, at the onset of clinical symptoms, and at the end stage of disease, which occurred after 164 ± 6 d (expressed as the mean ± standard deviation [SD]; $n = 5$). Skin samples from the *following five body regions of each hamster were analyzed:* the forelimb, the hindlimb, the abdomen, the back, and the head.

PrP27–30 could not be detected in any of the examined skin samples at 70 and 100 dpi from five animals each (Figure 1A and 1B). The earliest unambiguous signals for accumulation of the pathological prion protein PrP^{Sc} were found in skin samples from three out of five animals at 130 dpi in the late pre-clinical phase of incubation, corresponding to about 80% of the mean incubation period until terminal disease (Figure 1C, Western blot on the right-hand side, lanes S1, S2, S4, and S5). However, variable combinations of PrP^{Sc}-positive skin samples from different regions of the body were found at 130 dpi, indicating individual variation in the spread of infection to, or inhomogeneous distribution of PrP^{Sc} in skin tissue. Possibly, prion infection of the skin could have been detected more frequently in animals at 130 dpi, or at earlier pre-clinical stages of incubation, by using alternative methodologies such as the conformation-dependent immunoassay [19] or bioassays. At the onset of clinical symptoms, all of the analyzed skin specimens from all five examined hamsters displayed more or less strong signals for PrP27–30 (Figure 1D). At the terminal stage of scrapie, the positive signals for PrP27–30 become more intense, suggesting that accumulation of PrP^{Sc} takes place predominantly in relatively late stages of incubation (Figure 1E, lanes S1–S5). The weight of the tested skin samples ranged from approximately 40 to 100 mg as specified in the legend to Figure 1A–1E. In order to verify that the detected bands originated from PrP^{Sc}, a control experiment was performed: After deglycosylation with PNGaseF, the PrP27–30 bands showed an electrophoretic shift towards a single band at about 20 kDa, the molecular weight to be expected for the unglycosylated PrP27–30 form of 263K hamster scrapie (Figure 1F, lanes S1d–S5d). Control samples from mock-challenged age-matched hamsters consistently produced negative results (not shown). A time-scale displaying an overview of the time-points at which the p.o.-infected hamsters were tested for skin-associated PrP^{Sc} deposition in relation to the mean incubation period and the pre-clinical and clincal phases of incubation is provided in Figure 1G.

Location of PrP^{Sc} within the Skin

To determine where in the skin PrP^{Sc} accumulates, we investigated samples from the head, snout, forelimb, and abdomen of orally 263K scrapie–infected, terminally ill hamsters. As done previously when determining the route of PrP^{Sc} propagation to muscles [11], we used the paraffin-embedded tissue (PET) blot method to achieve a sensitive topographical localisation of disease-associated PrP in the skin. Using either Carnoy- or paraformaldehyde-fixed tissue

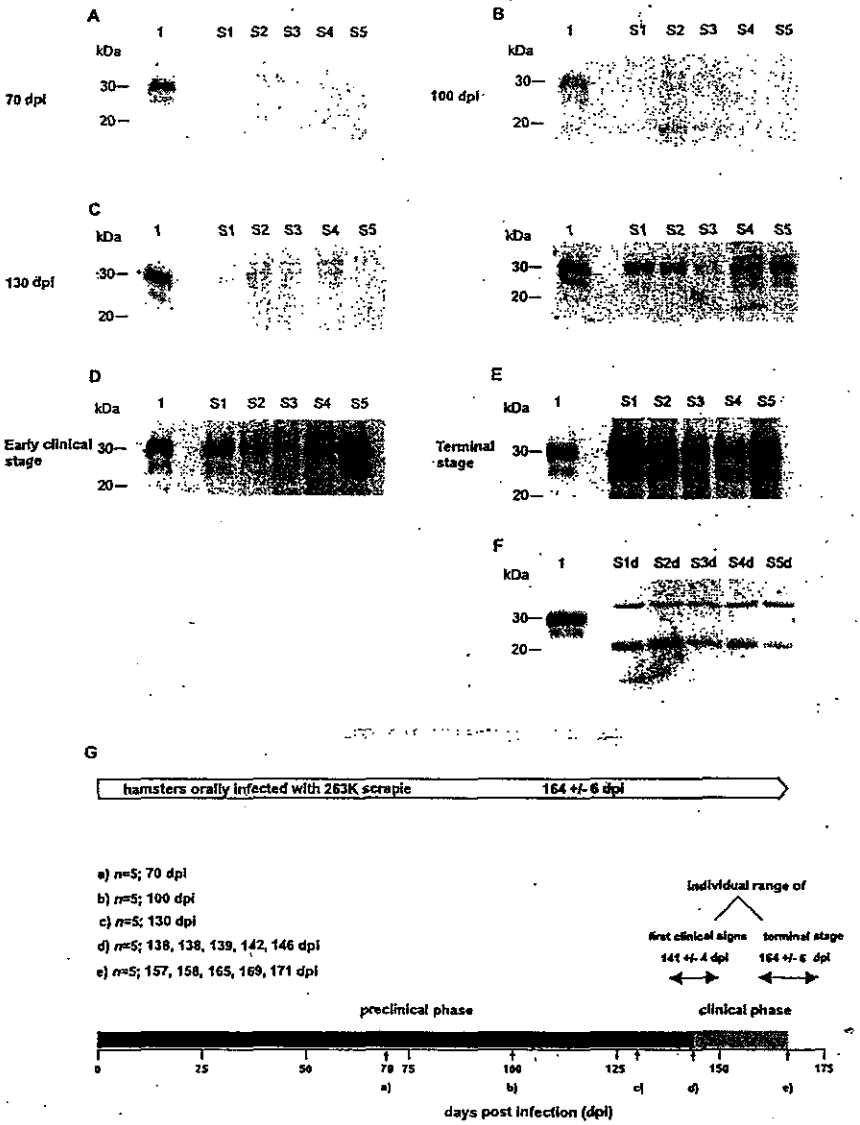

Figure 1. Time-Course of PrP^{Sc} Deposition in Skin Tissue

(A–E) Western blot detection of PrP27–30, the protease-resistant core of PrP^{Sc}, extracted from different skin samples of hamsters orally challenged with 263K scrapie and sacrificed at the following time-points after infection: (A) 70 dpi, (B) 100 dpi, (C) 130 dpi, (D) at the onset of clinical signs for scrapie (138–146 dpi), and (E) at the terminal stage of disease (157–171 dpi). Lanes with test samples: S1, skin sample from hindlimb; S2, skin sample from forelimb; S3, skin sample from back; S4, skin sample from abdomen; S5, skin sample from head. Lanes with control samples: 1, proteinase K–digested brain homogenate from terminally ill 263K scrapie hamsters containing 1×10^{-7} g brain tissue. Representative results are shown for each stage of incubation. Substantial individual variation was observed at 130 dpi, with two of five and three of five animals displaying findings as in (C) in the Western blot on the left-hand side or the Western blot on the right-hand side, respectively.

(F) Lanes S1d–S5d: Same samples as in S1–S5 of (E) but deglycosylated with PNGaseF.

(A–F) Amounts of tissue represented in lanes: (A) S1, 43 mg; S2, 52 mg; S3, 68 mg; S4, 58 mg; S5, 73 mg; (B) S1, 78 mg; S2, 44 mg; S3, 63 mg; S4, 67 mg; S5, 50 mg; ([C], Western blot on the left side) S1, 42 mg; S2, 76 mg; S3, 61 mg; S4, 58 mg; S5, 73 mg; ([C], Western blot on the right side) S1, 51 mg; S2, 63 mg; S3, 70 mg; S4, 87 mg; S5, 54 mg; (D) S1, 63 mg; S2, 68 mg; S3, 90 mg; S4, 50 mg; S5, 68 mg; (E) S1, 55 mg; S2, 73 mg; S3, 80 mg; S4, 88 mg; S5, 70 mg; (F) S1d, 12 mg; S2d, 14 mg; S3d, 19 mg; S4d, 12 mg; S5d, 20 mg.

(G) Time-scale displaying the mean incubation period and the pre-clinical and clinical phases of incubation of hamsters orally infected with 263K scrapie. Small vertical arrows indicate time-points at which animals were tested for PrP^{Sc} in skin samples.

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samples, PrP^{Sc} was detectable in (i) free nerve endings of the subepidermal plexus on the border of the epidermis to the dermis (Figure 2A, 2B, 2G, and 2H, arrows), (ii) fibres of the subepidermal, the deep cutaneous, and the subcutaneous plexus, (iii) fibres of the follicular neural network of the hair (circular and longitudinal fibres, Figure 2A, 2B, and 2G, arrowheads), (iv) the hair follicle isthmus (Figure 2G, rhombus), and (v) small intradermal striated fibres of mimic muscles (Figure 2A and 2B, asterisks). No PrP^{Sc} was detectable in keratinocytes, epidermal basal cells, fibroblasts of the dermal connective tissue, capillary blood vessels, outer root sheet cells of the hair, or the bulge region and the sebaceous gland, but PrP^{Sc} was present in nerve fibres of the sebaceous gland (not shown). Nerve fibres in the skin can be labelled by