These data probably indicate that insufficient lysine residues are modified to destroy antigenic determinants by treating L7/L12 with 500 molar excess. However its partial loss of activity suggests a modification in binding sites to the ribosomal particle, according to the molecular model proposed by Luer and Wong[17], which shows accumulation of lysine residues in the C-terminal region that would interact with the ribosomal particle[17]. On the other hand, elution volume from a Sephadex G100 column of succinylated protein (2750 molar excess, data not shown) do not differ appreciably from the one for native L7/L12, suggesting that chemical modification does not seem to be related to dimeric structure.

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10 Abbreviations used: HEPES, N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid; DTT, dithiothreitol; EDTA, ethylenediaminetetra-acetic acid, disodium salt.
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A new method for determining the microbial degradation of keratin in soils

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Summary. Keratin azure was incubated in soils in mesh bags of pore-size 5 μm, chosen to allow micro-organisms, but not soil animals access to the substrate. The non-degraded substrate was solubilized and the amount of dye remaining determined as a measure of keratin breakdown.

Keratin is a complex fibrous protein found in hair, hoof, horn and nails, which is colonized and degraded by soil micro-organisms, principally keratinophilic fungi[2]. Dermatophytes, pathogenic on man and animals, can survive as saprophytes on keratin, so it is important that degradation rates for this substrate in soil be accurately determined.

The technique described here involves use of keratin azure, a commercially available keratin substrate dyed with Ramazol Brilliant Blue R. The dye is covalently linked to the substrate, and release of the blue color is a measure of keratin degradation. Microbial degradation of keratin azure in vitro leads to the release of blue color into the medium, which can then be easily measured spectrophotometrically. This direct approach is not possible in soil studies, however, because of interference from soil color and possible adsorption of the liberated dye onto soil colloids and minerals; as a result, the amount of dye lost from a known weight of keratin azure following incubation in soil was determined in the method described here.

Keratin azure (Sigma, 0.01 g) was placed between 2 squares of polyester fabric (4×4 cm) (chosen to exclude soil animals, but not micro-organisms) of mesh size 5 μM and

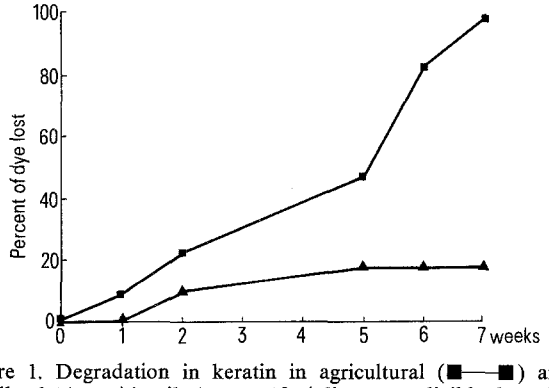

Figure 1. Degradation in keratin in agricultural (■——■) and woodland (▲——▲) soils (means of triplicates, negligible degradation occurred in autoclaved control). SD never exceeded ± 12%.

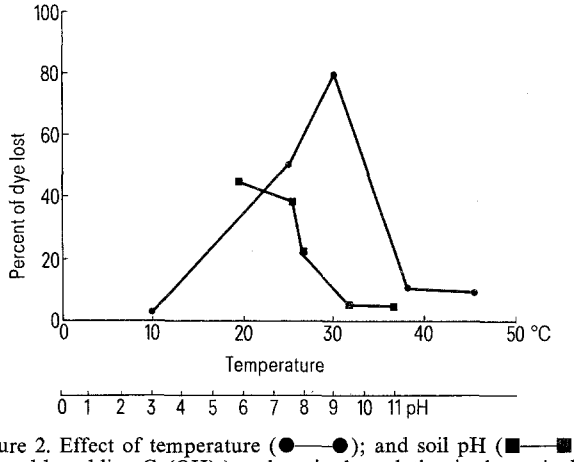

Figure 2. Effect of temperature (●——●); and soil pH (■——■) (altered by adding $Ca(OH)_2$) on keratin degradation in the agricultural soils (5 weeks incubation, means of triplicates). SD never exceeded ± 12%.

the edges of the material heat-sealed. The mesh bag was placed inside a plastic photographic slide (5 × 5 cm), from which the glass had been removed leaving a window (35 × 22 cm). The 2 halves of the slide were then pressed together and the sides fused by touching them against a hot plate.

The slides provided support for the mesh bags, making handling easy; they did not degrade over the incubation period nor did the plastic appear to inhibit microbial growth.

After incubation in soil the slides were removed and opened and any undegraded keratin azure was carefully removed from the inside surface of the mesh bags and transferred to a centrifuge tube. The substrate was repeatedly washed with deionized water and centrifuged until no further dye release occurred. The water was then removed and 10 ml of NaOH (10% w/v) added. Finally, the tube was heated in a boiling water bath until the keratin azure dissolved when the resultant blue color was determined spectrophotometrically at 595 nm, using NaOH as blank. Samples of keratin azure (0.01 g) were then solubilized in the same way to determine the amount of dye initially present in the substrate, so that the amount of dye lost could be calculated by subtraction. Variation in dye content occurs when different batches of keratin azure are used so dye-loss values should be carefully matched with initial dye values obtained using the same substrate batch. The keratin azure was not sterilized prior to incubation in soil as autoclaving is known to markedly denature keratin proteins[2], while isolation studies showed that the fresh substrate was free of keratinophilic micro-organisms.

Keratin degradation was studied in an agricultural soil (previous crop potatoes; pH, 5.7; total C, 5.5%; total N, 0.7%), and a deciduous woodland soil (pH, 4.9; total C, 9.8%; total N, 0.9%). Slides were placed in 200 g of soil in triplicate, and incubated in sterile polythene bags at 20% v/w water content (maintained throughout) at 25 °C. The polythene bags were closed with an elastic band so as to leave a small hole to allow for gas exchange. Slides were also placed in soil which had been autoclaved on 3 successive occasions at 120 °C for 20 min to act as controls.

Keratin breakdown was much more rapid in the agricultural than in the woodland soil (fig. 1), despite the fact that it had not recently supported livestock, and was thus unlikely to have a high keratin content. Degradation decreased with increasing pH and was optimal at 30 °C (fig. 2), and was negligible in autoclaved soil.

Rapid keratin degradation is generally thought to occur in soils containing large amounts of the substrate[3], although even soils apparently lacking keratin contain keratinophilic fungi[4]. Keratin degradation in the woodland soil was not however, stimulated by the addition of ball-milled keratin; wool or human hair (0.5–200 g soil) to the soil 6 weeks prior to insertion of the slides. Unlike other complex substrates the addition of keratin to soil appears not to rapidly stimulate micro-organisms capable of degrading it.

This technique is currently in use in this laboratory to determine the microbial degradation of keratin in soils frequented by humans (e.g. parks and beaches) and animals, with a view to determining which soils are likely to harbor both the substrates and associated dermatophytes.

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Incorporation of uridine diphospho-N-acetyl-D-glucosamine in the resting sporangium wall of *Synchytrium endobioticum*

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Summary. The microfibrils that constitute the walls of *Synchytrium endobioticum* resting sporangia are laid down at the periphery of developing walls and are packed in discrete orientation. Incorporation of uridine diphospho-N-acetyl-D-glucosamine, a precursor of chitin, is restricted to the periphery of the developing wall of the resting sporangium.

A major problem in the control of potato wart disease, incited by *Synchytrium endobioticum* (Schilb.) Perc., is the persistence of resting sporangia (meiosporangia) in the soil[2]. These propagules are known to be viable for 37 years[3]. The basis of such longevity, although not clear, putatively lies in the make up of the sporangium and its wall. Recent observations on the developing walls of sporocysts of a related species, *S. mercuriales* have revealed a complex orientation of microfibrillar material[4] similar to chitin. We report in this short communication a) the presence of microfibrils showing patterns of orientation similar to those observed on other chitinous structures[5], and b) evidence for incorporation of uridine diphospho-N-acetylglucosamine (UDP-GlcNAc), a precursor of chitin, into the walls of developing sporangia.

Materials and methods. For electron microscopy, pieces of fresh potato wart tissue were put into a fixative mixture of paraformaldehyde and glutaraldehyde[6] in phosphate buffer pH 7.2 at 23 °C for 1 h. The samples were washed thoroughly in cold (4 °C) buffer and treated with 1% OsO_4 in the same buffer. Dehydration was accomplished through an ethanol series for final embedding in Spurr's medium. Ultrathin sections were cut with a Porter-Blume ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a Zeiss 9S electron microscope.

Small (0.5 cm^3) slivers of fresh potato wart tissue cut from field grown potatoes cv. Arran Victory (susceptible to *S. endobioticum* European race 2), were washed in distilled water and treated with UDP-(^{3}H) GlcNAc (0.01 mCi/ml). Samples were taken out at 3, 6, 12 and 48 min and fixed in