tion of fixative; perfusion of these small fish was not attempted. The brain was then quickly excised, placed in Rossmans' fixative precooled to −35 °C and kept at that temperature for 24 h. Brains were then passed through 3 changes of ethanol at 4 °C each of 1 h duration. Following this, specimens were cleared in xylene and embedded in wax. This method is designated 'experimental' in the table. Sections from all blocks were cut at 7 µm, floated on water (standard method) or alcohol (experimental method) and stained by methods listed in the table. Hydration was avoided wherever possible in 'experimental' methods. A brain was fixed for electron microscopy[17] while the fish was anaesthetized.

Results. In the brain of fish fixed and stained by methods used by others the parasites were surrounded by vacuolated cells which were cytochemically inactive except for alkaline phosphatase (table). These results confirm the earlier work[3,5,8,10,11].

In contrast, cells around parasites stained strongly (fig. 1) in sections prepared and stained by methods which minimize post-mortem change and the loss of soluble or diffusible substances (table). These cells were seen also at some distance from the parasites, especially in the meninges and under the ependyma. The PAS reaction was markedly reduced when aqueous solutions of periodic acid were used.

Electron microscopy showed parasites to be in contact with neuroglia[17] and extensions of these cells could be very thin (fig. 2). Some neuroglial cells were small (fig. 2), had little cytoplasm, contained glycogen, long profiles of endoplasmic reticulum and mitochondria, but little else, whereas large cells were filled with vesicles (fig. 2), subdivided into groups by the scanty cytoplasm; also present were glycogen, Golgi bodies, mitochondria and lipid inclusions. Cells existed that were intermediate in size and cytology between these extremes.

No equivalent PAS-rich cells were detected in the uninfected fish although serial sections of the whole brain were studied.

Discussion. Anaesthesia was used in the experimental method because post-mortem changes in brain are well known to be rapid. The avoidance of incubation of tissue in aqueous solutions permits the demonstration of cytochemically rich cells where otherwise unreactive, vacuolated cells were seen. By their staining reaction, such cells could be detected elsewhere in the infected brain, in addition to those in contact with the parasites. The less-differentiated cells resemble microglia[20] but certain identification of neuroglia in fish cannot be made; the need for a systematic study of the pathology of fish nervous tissue has been recognised[14]. 2 sites of origin account for cells which respond to brain lesions[20]: astrocytes and oligodendrocytes arise from ectodermal precursors beneath the ependyma, whereas microglia have their origin in the mesoderm. Phagocytes from the blood following vascular injury may further contribute to the cellular response.

To be determined next is the origin of the cells that invest the parasites and appear to buffer it from neurons.

1 Acknowledgment. M.R.L. Johnston advised me during the course of this work.
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Studies on the modification of *Escherichia coli* ribosomal protein L7/L12 by succinic anhydride[1]

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Summary. Lysine modification by increasing quantities of succinic anhydride in the *Escherichia coli* ribosomal protein L7/L12 produces loss of its ability in reconstitution of elongation-factor-G-dependent GTP hydrolysis and polyphenylalanine synthesis activities, showing lower antigenicity and loss of antigenic determinants.

The *Escherichia coli* L7/L12 ribosomal protein may be removed from ribosomes quite specifically by ethanol-ammonium chloride[2], giving particles (cores) unable to support most of elongation-factor-dependent functions[3,4]. These particles efficiently rebind L7/L12, achieving the normal stoichiometry of 4 L7/L12 monomers in dimeric structure per ribosome[2,5].

Chemical modification of amino acid side chains within proteins has yielded extensive information about the role of particular amino acid residues and maintenance of native structure and antigenicity[6,7]. Succinic anhydride is able to react with lysine and tyrosine, but only lysines remain succinylated over pH 5.0, replacing a positive by a negative charge[8]. In the present study the L7/L12 modification by succinic anhydride has been examinated in relation to its functional activity and antigenicity.

Materials and methods. Escherichia coli strain MRE 600 was collected in logarithmic phase and ribosomes prepared as previously described[9]. Ribosomal protein L7/L12 was obtained from ribosomes by treatment with 1 M ammonium chloride and 50% v/v ethanol[2]. The isolated protein dissolved in 0.05 M HEPES[10], 0.02 M $MgCl_2$, 0.0005 M DTT

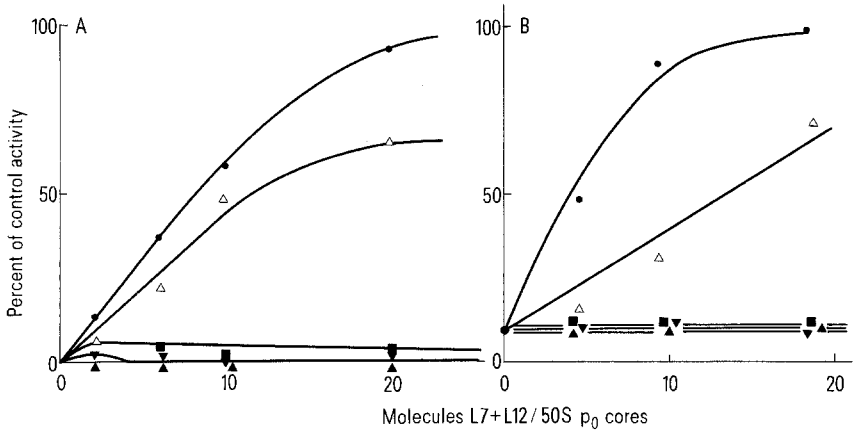


Figure 1. Effect of the addition of treated L7/L12 to P_0 50S cores on polyphenylalanine synthesis (A) and elongation-factor-G-dependent GTP hydrolysis (B). The activity is expressed as a percentage of the untreated control (50S+30S). The 100% activity was 90 moles of GTP hydrolyzed per mole of ribosomes for GTP hydrolysis and 2.2 moles of ($3H^3$)phenylalanine incorporated per mole of ribosomes for polyphenylalanine synthesis. Protein added: L7/L12 treated with 5 (●), 500 (△), 2750 (■), 5000 (▼) and 50,000 (▲) molar excess succinic anhydride.

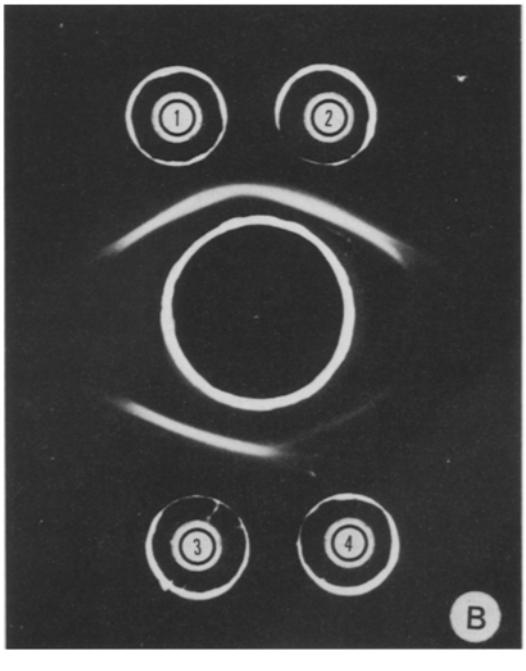
buffer, pH 8.2, at 0.23 mg/ml, was treated with succinic anhydride from 5 to 50,000 molar excess[11]. Immediately after treatment, samples were dialyzed overnight against 2000 vol. of 0.5% v/v acetic acid at 4 °C. Synthesis of polyphenylalanine was performed as already described[9] and elongation-factor-G-dependent GTP hydrolysis was carried out as described by Ballesta et al.[12], both in protein deficient 50 S ribosomal particles (cores P_0)[4].

Immunodiffusion was performed in 0.75% w/v agarose made in 0.01 M Tris-HCl buffer, pH 7.4, 0.15 M NaCl, using rabbit antiserum against L7/L12[13]. Polyacrylamide slab gel electrophoresis was performed at a concentration of 10% T[14] with a stacking gel of 4% T, both 5.25% C in the Davis discontinuous system[15]. The gel was run at 50 V and stained with Coomassie Brilliant Blue G 250 in perchloric acid[16].

Results and discussion. After treatment of L7/L12 *E. coli* ribosomal protein with succinic anhydride at a 5 to 50,000 reagent-to-protein molar ratio, the reconstitution of the activity of the L7/L12 deficient ribosomal particles with the modified protein was studied. As shown in figure 1, reconstitution of polyphenylalanine synthesis and elongation-factor-G-dependent GTP hydrolysis is lost in both cases; only partially when the L7/L12 was treated with a 500 molar excess, but completely when the ratio of succinic anhydride to protein is 2750 molar or more.

Gel diffusion analysis with rabbit anti L7/L12 antiserum demonstrated that the succinylated protein shows the same reactivity as the native molecule up to 2750 molar excess reagent. This modified protein presents lower antigenicity and the control protein shows a big spur (fig. 2) indicating loss of antigenic determinants in the modified protein. In contrast to the above observation, lines of identity were observed between 500 molar excess-treated and native L7/L12 (fig. 2).

The relation between alteration of reconstitution activities, elongation-factor-G-dependent GTP hydrolysis and polyphenylalanine synthesis, and antigenic changes recognized by antinative antiserum in succinylated L7/L12, suggests that structural changes have taken place in the native molecule as a result of reaction with a 2750 molar excess of succinic anhydride. The small loss of reconstitution activity with 500 molar excess treated protein and its antigenic identity with the native protein can be related to the small changes in electrophoretic mobility in polyacrylamide gel that this treated protein shows (fig. 2) in comparison with the 2750 molar excess modified protein.



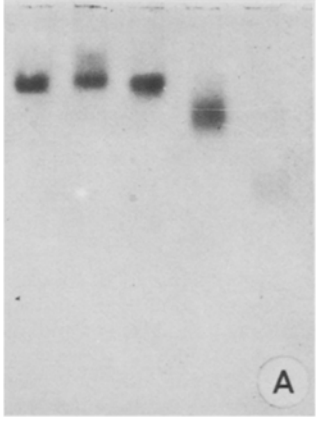


Figure 2. Succinylated L7/L12. *A* Polyacrylamide gel electrophoresis. The proteins are from left to right, L7/L12 untreated, 5, 50, 500 and 2750 molar excess treated proteins. *B* Double immunodiffusion test. The peripheral wells contained, L7/L12 control (1 and 3) 500 and 2750 molar excess treated protein (2 and 4). The central well contains anti-L7/L12 antiserum.

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

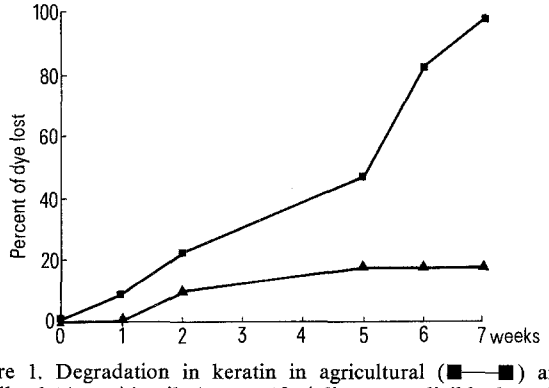

Figure 1. Degradation in keratin in agricultural (■——■) and woodland (▲——▲) soils (means of triplicates, negligible degradation occurred in autoclaved control). SD never exceeded ± 12%.

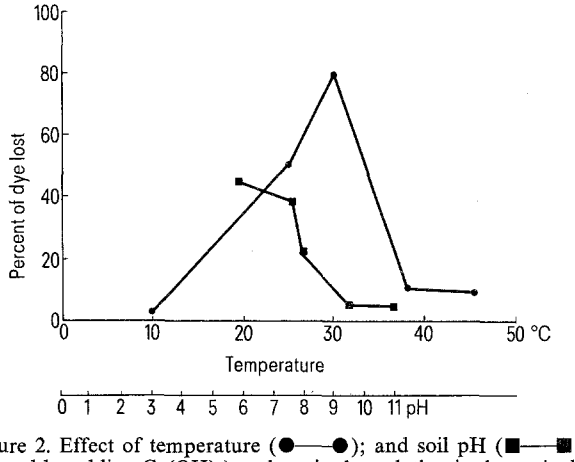

Figure 2. Effect of temperature (●——●); and soil pH (■——■) (altered by adding $Ca(OH)_2$) on keratin degradation in the agricultural soils (5 weeks incubation, means of triplicates). SD never exceeded ± 12%.