Use of Collagenase in the Characterization of Pseudocœlomic Membranes of *Ascaris lumbricoides*

THE longitudinal muscle cells of *Ascaris lumbricoides* are enclosed in sheaths that form part of the system of pseudocœlomic membranes covering the internal organs of nematodes. The nature of these membranes is obscure, though Monné[1] demonstrated that they stain like collagen. Collagenous structures previously described in *Ascaris* are the cuticle and the basal lamella of the intestine. The collagenous nature of the former was inferred from the results of X-ray studies[2] and paper chromatographic analysis[3], that of the latter from X-ray studies only[4]. Neither material showed any trace of a banded structure in electron micrographs, nor did X-ray diffraction photographs suggest the presence of long spacings.

As the membranes are difficult to isolate in bulk for physical and chemical analysis, it was decided to test for the presence of collagen by using collagenase. Doubts have been expressed about the specificity of the collagenases present in *Clostridium welchii* and *C. histolyticum* filtrates, but Seifter *et al.*[5] have shown that the purified collagenase of *C. histolyticum* degrades only collagen and gelatin. Robb-Smith[6], working with mammalian tissues, showed the specificity of *C. welchii* collagenase under defined conditions, particularly when antisera are used to neutralize other enzymes present in the filtrate. As *C. histolyticum* collagenase was unobtainable, it was decided to follow the technique of Robb-Smith.

Enzyme tests were carried out using a Wellcome Physiological Research Laboratory *C. welchii* filtrate (*B* 637 *C*) with the following specifications: collagenase, 52 *k* units/mgm. (22 *Q* units/mgm.); lecithinase, 4·3 units/mgm.; protein, N 3·6 per cent; hyaluronidase, 8 units/mgm., and a small quantity of λ, a protease of unspecified concentration. A refined antiserum (*RX* 5755/36) of the following concentrations was also supplied: anti-collagenase, 1·5 units/ml.; anti-lecithinase, 220 units/ml.; anti-hyaluronidase, 650 units/ml.; anti-λ, 6,000 units/ml., which should be sufficient to neutralize the λ in *B* 637 *C*.

A solution containing approximately 1,560 *k* units (660 *Q* units) of collagenase was obtained by adding 30 mgm. of *B* 637 *C* to 1·5 ml. of *RX* 5755/36 in 100 ml. of Palitisch's[7] borate buffer at *p*H 6·8. Material was fixed in Carnoy's fluid, and paraffin sections were cut at 10μ. After the removal of paraffin, and hydration, the slides were incubated at 37° C. either in collagenase solution or in buffer solution as control. At intervals, slides were removed, washed, 'Celloidinized', and stained by the Gomori[8] modification of the Bielschowsky-Maresch silver method for collagen.

After 6 and 10 hr., the material appeared unchanged; but 12 hr. and longer incubation caused digestion not only of the basal lamella of the intestine but also of the sheaths surrounding the muscle cells. After 16 hr. incubation, the cuticle had completely disappeared apart from the external cortical layer. Bird[9] has presented evidence that this layer is a tanned protein, so its resistance is not surprising. Digestion of the basal lamella of the intestine and the internal portion of the cuticle, as well as of the sheaths surrounding the muscle cells, strongly suggests that the sheaths are collagenous.

Studies with the electron microscope are envisaged for the future.

Now that the specificity of the collagenases of clostridial filtrates has been established[5,6], its use could conveniently form a diagnostic test for collagenous materials.

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Isolation of a Phytoalexin from *Pisum sativum* L.

THE presence of an antifungal 'principle (or principles)' produced by pods of *Phaseolus vulgaris* L. in response to infections with *Sclerotinia fructicola* (Wint.) Rhem. has been demonstrated on the basis of biological assays by Muller[1,2]. We now report the isolation of an antifungal compound produced in similar hypersensitivity studies using pods of *Pisum sativum* L., and describe some of its chemical, physical and biological properties.

The biological conditions provided for the interaction of host and fungus were similar to those reported by Muller[1], namely, the inoculation of the endocarps of detached, opened pods with a fungal spore suspension and the recovery of the drops of liquid ('diffusate') remaining on the tissue after suitable incubation periods. Quantitative bioassays, based on the inhibition of spore-germination in seeded agar blocks, with *S. fructicola* as test organism, were used to determine antifungal activity.

The active material was extracted from the crude diffusate by light petroleum (b.p. 55–60° C.); the residue was not antifungal. When concentrated extracts were fractionated using paper chromatography (solvent *n*-propanol/water, 20 : 80 v/v), the fraction with an R_F 0·6–0·7 contained most of the biological activity. An antifungal compound from this fraction has been obtained since in crystalline form by repeated re-crystallization from light petroleum. It is sparingly soluble in water but is soluble in a wide range of organic solvents. Microanalyses and molecular weight determinations are consistent with the formula $C_{17}H_{14-16}O_6$. Other physical properties include m.p. 61° C.; ultra-violet absorption maxima in water at 307 mμ (log ε 3·85) and 286 mμ (log ε 3·7); molecular optical rotation $[M]^{200}_{578\ m\mu}$ = 880 (*c.* = 0·1 in ethanol). The substance is stable in alkaline or neutral solution, but