

tracer molecule in the study of protein transport across cellular membranes<sup>3-5</sup>.

Adult *Taenia hydatigena* were obtained from naturally infected dogs by removal from the small intestine, and were washed in phosphate buffer at pH 6.5. The tracer protein employed was horseradish peroxidase and the procedure followed was that of OSBORNE and MILLER<sup>6</sup> with appropriate modifications. After washing, the live specimens were placed in concentrations of 0.1, 0.5 and 1.0% horseradish peroxidase in phosphate buffer at pH 6.5 for periods of 30 min to 48 h. All the experiments were performed at 24°C. For visualizing the sites of peroxidase uptake, the animals were removed from the incubation medium at different time intervals and rinsed in phosphate buffer. Different regions of the worm were sliced in ice-cold 4% buffered neutral formaldehyde and kept in the same solution for 4 h fixation. The different regions of the worm were washed thrice with ice-cold 10% sucrose solution at 30 min intervals. Subsequently the material was embedded in gelatin and frozen sectioned. The sections were washed repeatedly in phosphate buffer (pH 7) and 9 ml of benzidine reagent (0.3% benzidine in phosphate buffer at pH 7) was added with gentle shaking. After 2 min, 2 ml of 0.3% hydrogen peroxide was added and the sections shaken vigorously at room temperature for 10-30 min. The sections were dehydrated, cleared in xylene and mounted in Canada balsam. Regions taking up peroxidase turned dark brown on treatment with benzidine reagent<sup>7</sup>. Control animals were incubated in a similar manner with the omission of peroxidase from the

medium and fixed in the same manner as the experimental animals.

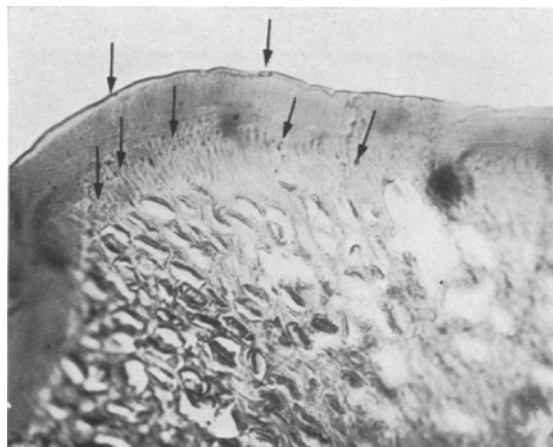
In the experimental animals, the tegument of the neck region and gravid proglottides did not show any evidence of peroxidase uptake even after incubation for 48 h using different concentrations of the incubation medium. In the region of the mature proglottides, the tegument showed a positive reaction at 0.1% peroxidase concentration after 24 h of incubation. In 0.5% concentration, granules were observed in the tegument even at 12 h. In a 1.0% concentration, the uptake of peroxidase was faster, the tegument showed a positive reaction after 6 h of incubation (Figure). Control animals did not show any positive benzidine reaction in any region of the body.

It may be seen from the foregoing account that the peroxidase uptake occurs only in the mature proglottides region, suggesting that this region alone is involved in the protein sequestration from the host intestine. Although the absence of peroxidase uptake in the region of gravid proglottides may be correlated with the stabilized nature of its protein component of the tegument<sup>8</sup>, the factors which restrain the entry of peroxidase through the tegument of the neck region, the protein component of which is unstabilized and similar to that of mature proglottides<sup>9</sup>, are not clear at present.

**Zusammenfassung.** Die Durchlässigkeit der Kutikula verschiedener Körperabschnitte von *Taenia hydatigena* für Eiweissmoleküle wurde mittels Meerrettich-Peroxidase untersucht. Dabei wurde nachgewiesen, dass die Kutikula von *T. hydatigena* nur im Bereich der reifen Proglottiden für Peroxidase durchlässig ist. Dies lässt die Annahme zu, dass einzig in diesem Körperabschnitt von *T. hydatigena* die Resorption von Eiweiss aus dem Darm des Wirtes erfolgt.

S. MUTHUKRISHNAN<sup>10</sup>

Department of Zoology, University of Madras,  
Madras-600005 (India), 12 September 1974.



Transverse section passing through the mature proglottid of *Taenia hydatigena*. Arrows indicate the location of the peroxidase granules in the tegument and sub-tegumental regions.

### ***Penicillium lilacinum*: Its Tolerance to Cadmium**

A number of metal-resistant fungi were isolated from farm land polluted by mine drainage<sup>1,2</sup>. This experiment was carried out to find the distribution of the fungi in the land polluted by cadmium and the tolerance of the fungi to cadmium.

**Materials and methods.** Soil and water were collected from Sasagadani mine field<sup>3</sup> and its neighborhood as shown in the Table. Cadmium contents in the collected samples were measured using atomic absorption spectrophotometer, and fungi were isolated from the samples using potato sucrose agar (PSA)-rosebengal-streptomycin

medium or the medium containing 1,000 ppm of cadmium<sup>4</sup>. Tolerance of the fungi to cadmium were found by the mycelial growth on PSA medium containing cadmium at a concentration of 10,000 ppm as a maximum and 1,000

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<sup>2</sup> K. TATSUYAMA, H. EGAWA, H. YAMAMOTO and H. SENMARU, Transact. mycol. Soc. Japan 16, 69 (1975).

<sup>3</sup> Shimane Pref., Japan.

<sup>4</sup> Cadmium chloride was used.

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Places for collecting of the samples polluted by cadmium and fungi isolated from the samples

Places	Samples	Cadmium contents (ppm)	Cd-resistant fungi	Tolerance to cadmium (ppm)
Neighborhood of closed refinery	Soil ( $\times 2$ ) <sup>a</sup>	23,15 <sup>b</sup>	<i>Penicillium lilacinum</i>	10,000
			<i>Paecilomyces</i> sp.	10,000
			<i>Synnematium</i> sp.	10,000
			<i>Cephalosporium</i> sp.	4,000
	Standing water	0,15	<i>Penicillium lilacinum</i>	10,000
			<i>Penicillium waksmani</i>	8,000
			<i>Penicillium fumiculosum</i>	2,000
			<i>Penicillium</i> sp.	2,000
River from the mine field	Mud ( $\times 6$ )	6,05	<i>Trichoderma</i> sp. ( $\times 2$ ) <sup>c</sup>	4,000–2,000
			<i>Penicillium lilacinum</i>	10,000
			<i>Paecilomyces</i> sp.	2,000
			<i>Trichoderma</i> sp. ( $\times 5$ )	8,000–1,000
	Water ( $\times 2$ )	0,14	<i>Helminthosporium</i> sp.	4,000
			<i>Doratomyces</i> sp.	4,000
			<i>Cladosporium</i> sp.	6,000
			<i>Trichoderma</i> sp. ( $\times 2$ )	4,000
Paddy field (polluted)	Soil ( $\times 5$ )	2,37	<i>Penicillium lilacinum</i> ( $\times 6$ )	10,000
			<i>Trichoderma</i> sp.	8,000
			<i>Helminthosporium</i> sp.	1,000
			<i>Cephalosporium</i> sp.	4,000
	Soil ( $\times 4$ )	0,42	<i>Curvularia lunata</i>	6,000
			<i>Microascus</i> sp.	1,000
			<i>Fusarium</i> sp.	6,000
			<i>Trichoderma ligurosus</i> ( $\times 2$ )	6,000–1,000
Paddy field (little polluted)	Soil ( $\times 4$ )	0,42	<i>Trichoderma</i> sp.	4,000
			<i>Helminthosporium</i> sp. ( $\times 2$ )	2,000–1,000
			<i>Microascus</i> sp.	1,000

<sup>a</sup> Number of samples. <sup>b</sup> The average value. <sup>c</sup> Number of strains.

ppm as a minimum. The tolerance of 40 isolates, obtained from the land by the use of the medium containing 1,000 ppm of cadmium, was evaluated.

**Results and discussion.** A number of fungi isolated from the polluted samples by the use of the PSA-rosebengal-streptomycin medium showed a decrease compared with those from the soil outside the farm. The ratio of the number of the fungi isolated from the samples using the medium containing 1,000 ppm of cadmium to the number of those isolated by the use of the medium without cadmium were raising according to the increase of cadmium contents in the samples. As shown in the Table, *Penicillium lilacinum* accounts for 23% of all the fungi isolated by the use of the medium containing 1,000 ppm of cadmium, and seems to be a strong resistant fungus to cadmium. Judging from these results, *P. lilacinum* may be a dominant species in land polluted by cadmium, and it was presumed that *P. lilacinum* is an indicator fungus in the biological investigation of the soil pollution.

In addition to the fungus, 2 isolates of *Paecilomyces* sp. and *Synnematium* sp. showed resistance to 10,000 ppm

of cadmium. *Penicillium waksmani* and an isolate of *Trichoderma* sp. showed resistance to 8,000 ppm of cadmium.

**Summary.** *Penicillium lilacinum*, one of the fungi isolated from farm land continuously irrigated from the mine fields, may be a dominant species in the land polluted by cadmium, so it was presumed that *P. lilacinum* is an indicator fungus in the biological investigation of the soil pollution.

K. TATSUYAMA, H. EGAWA, H. SENMARU,  
H. YAMAMOTO, S. ISHIOKA<sup>5</sup>  
T. TAMATSUKURI<sup>5</sup> and K. SAITO<sup>5</sup>

Faculty of Agriculture, Shimane University,  
Matsue 690 (Japan), and  
Shimane-ken Public Health Laboratory,  
Matsue 690 (Japan), 25 February 1975.

<sup>5</sup> Shimane-ken Public Health Lab., Matsue 690 (Japan).

## Serum Protein Pattern of Mice During Infection with Single and Repeated Doses of *Ancylostoma caninum* Larvae

*Ancylostoma caninum* is one of the most pathogenic canine hookworm causing anaemia; the larvae also infect man cutaneously, producing clinical symptoms of cutaneous larva migrans or creeping eruption<sup>1-4</sup>, thereafter they may migrate to the lungs<sup>5,6</sup> and even appear in the sputum<sup>7</sup>. Significant alterations in the serum protein following different hookworm infections have been re-

ported in man<sup>8-10</sup>, dog<sup>11,12</sup> and other experimental hosts<sup>13</sup>. The understanding of zoonosis of *A. caninum*, and also of the immune responses induced by the infective larvae of Ancylostomes in their normal hosts (man and dog) during initial periods of infection within the tissues, can be appreciated in such experimental hosts (Swiss albino mice) where the larvae do not develop further<sup>14</sup>.