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 (×2)[a]	23,15[b]	*Penicillium lilacinum*	10,000
			Paecilomyces sp.	10,000
			Synnematium sp.	10,000
			Cephalosporium sp.	4,000
	Standing water	0,15	*Penicillium lilacinum*	10,000
			Penicillium waksmani	8,000
			Penicillium fumiculosum	2,000
			Penicillium sp.	2,000
			Trichoderma sp. (×2)[c]	4,000–2,000
River from the mine field	Mud (×6)	6,05	*Penicillium lilacinum*	10,000
			Paecilomyces sp.	2,000
			Trichoderma sp. (×5)	8,000–1,000
			Helminthosporium sp.	4,000
			Doratomyces sp.	4,000
			Cladosporium sp.	6,000
	Water (×2)	0,14	*Trichoderma* sp. (×2)	4,000
Paddy field (polluted)	Soil (×5)	2,37	*Penicillium lilacinum* (×6)	10,000
			Trichoderma sp.	8,000
			Helminthosporium sp.	1,000
			Cephalosporium sp.	4,000
			Curvularia lunata	6,000
			Microascus sp.	1,000
			Fusarium sp.	6,000
Paddy field (little polluted)	Soil (×4)	0,42	*Trichoderma ligurosum* (×2)	6,000–1,000
			Trichoderma sp.	4,000
			Helminthosporium sp. (×2)	2,000–1,000
			Microascus sp.	1,000

[a] Number of samples. [b] The average value. [c] 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 form 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 waskmani* 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.

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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[1–4], thereafter they may migrate to the lungs[5,6] and even appear in the sputum[7]. Significant alterations in the serum protein following different hookworm infections have been reported in man[8–10], dog[11,12] and other experimental hosts[13]. 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[14].

Electrophoretic analysis of serum protein of mice infected with various doses of *A. caninum* larvae

Dose of infection		Percentage of serum protein components						
		Albumin	α-1-globulin	α-2-globulin	β-globulin	γ-globulin	Total globulins	A/G ratio
Uninfected control		41.78 ± 0.90	4.18 ± 0.20	14.16 ± 0.61	30.91 ± 0.74	7.28 ± 0.49	56.67 ± 0.94	0.74 ± 0.034
Infected groups								
1	250 larvae	33.49[a] ± 2.80 ($p < 0.025$)	3.88[b] ± 0.31	16.48[b] ± 1.03	40.02[a] ± 2.56 ($p < 0.005$)	4.95[a] ± 0.52 ($p < 0.005$)	65.33[a] ± 2.97 ($p < 0.025$)	0.52[a] ± 0.058 ($p < 0.005$)
2	500 larvae	32.61[a] ± 1.79 ($p < 0.001$)	4.97[b] ± 0.41	17.52[b] ± 1.98	38.79[a] ± 2.20 ($p < 0.005$)	5.26[a] ± 0.42 ($p < 0.01$)	66.54[a] ± 3.27 ($p < 0.01$)	0.49[a] ± 0.033 ($p < 0.001$)
3	1000 larvae	33.09[a] ± 1.74 ($p < 0.001$)	4.25[b] ± 0.43	14.03[b] ± 0.73	42.28[a] ± 1.43 ($p < 0.001$)	5.21[a] ± 0.65 ($p < 0.025$)	65.77[a] ± 0.58 ($p < 0.001$)	0.51[a] ± 0.036 ($p < 0.001$)
4	2000 larvae	28.08[a] ± 1.43 ($p < 0.001$)	3.74[b] ± 0.26	15.46[b] ± 0.99	46.95[a] ± 2.07 ($p < 0.001$)	4.27[a] ± 0.52 ($p < 0.001$)	70.42[a] ± 2.16 ($p < 0.001$)	0.40[a] ± 0.029 ($p < 0.001$)
5	4000 larvae	21.05[a] ± 1.03 ($p < 0.05$)	2.99[b] ± 0.84	17.94[b] ± 2.90	48.55[a] ± 4.20 ($p < 0.05$)	6.41[b] ± 0.71	75.89[a] ± 0.56 ($p < 0.05$)	0.28[a] ± 0.023 ($p < 0.05$)
Immunized groups								
6	500 + 500 larvae	30.17[a] ± 1.88 ($p < 0.001$)	5.31[b] ± 0.52	17.38[a] ± 1.13 ($p < 0.025$)	38.68[a] ± 1.39 ($p < 0.001$)	6.90[b] ± 0.64	68.33[a] ± 1.96 ($p < 0.001$)	0.45[a] ± 0.042 ($p < 0.001$)
7	500 + 500 + 1000 larvae	34.15[a] ± 1.82 ($p < 0.005$)	4.75[b] ± 0.42	16.87[b] ± 1.23	39.35[a] ± 1.04 ($p < 0.001$)	4.54[a] ± 0.50 ($p < 0.005$)	65.51[a] ± 2.01 ($p < 0.001$)	0.53[a] ± 0.046 ($p < 0.005$)
8	250 + 500 + 1000 + 2000 + 4000 larvae	33.03[a] ± 2.13 ($p < 0.05$)	4.28[b] ± 0.24	16.06[b] ± 1.13	42.96[a] ± 2.13 ($p < 0.05$)	3.68[a] ± 0.43 ($p < 0.05$)	66.98[a] ± 2.55 ($p < 0.05$)	0.51[a] ± 0.060 ($p < 0.05$)

Average relative percentage of serum protein components during a 30 day infection period. All values except A/G ratio are expressed in average relative percentages with standard errors. [a] Statistically significant values. [b] Not significant values.

Recently BHOPALE and JOHRI[15] reported a decrease in the albumin and an increase in the β-globulin in serum of mouse infected with *A. caninum* larvae. The present communication provides an objective effort to determine alterations in serum protein of mice infected with single and repeated doses of *A. caninum* larvae.

Material and method. Infective *A. caninum* larvae were obtained from faeces by the method of SEN et al.[16].

Two experiments were conducted with Swiss albino mice of either sex (body weight 20–23 g) infected per os. In experiment No 1, single doses (250, 500, 1000, 2000 and 4000 larvae) were given to 5 different groups of mice, 20 in each. In experiment No. 2, repeated doses (500 + 500, 500 + 500 + 1000 and 250 + 500 + 1000 + 2000 + 4000 larvae) were given at weekly intervals to 3 different groups of mice, 20 in each. A group of 20 mice was kept as uninfected control. Two mice from each group were sacrificed and serum samples were separated from the blood collected by cardiac puncture, from day 3 at regular intervals of 3 days upto 30th day from mice of experiment No. 1 and similarly from those of experiment No. 2 after the last infection.

Results from a 30-day period of each infected group were compared with those of the control group, and among themselves also taking the last dose of the repeated infection as the basis for comparison with the single dose of infection as follows:

Groups	Experiment No. 1 (single dose)	Experiment No. 2 (repeated dose)	Control (uninfected)
1	500 larvae	500 + 500 larvae	no larvae
2	1000 larvae	500 + 500 + 1000 larvae	
3	4000 larvae	250 + 500 + 1000 + 2000 + 4000 larvae	

Serum samples were processed electrophoretically on Whatmann chromatographic paper No. 1 using barbitone buffer (pH 8.6), aqueous bromophenol blue and densitometer for scanning. The values of globulins were multiplied with 1.6 (correction factor[17]).

Results and discussion. In experiment No. 1, infected mice from all the groups survived for 30 days except those with a dose of 4000 larvae which survived for 6 days only. There was a significant increase in the β-globulin which was at the cost of albumin eventually resulting in the increase of total globulins and the decrease in the A/G ratio. There was no significant variation in the α-1 and α-2 globulins. The significant decrease in γ-globulin was recorded in all the infected groups, except in the one infected with 4000 larvae where it remained uneffected (Table). Statistical analysis showed a significant negative correlation between the infective dose and

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decrease in albumin level ($p < 0.01$) and a positive correlation with the increase of the β-globulin ($p < 0.05$), indicating that serum protein alterations took place according to the increasing dose levels.

In experiment No. 2, two animals from the group infected with a challenge dose of 4000 larvae died on the 24th day. Comparing with the control, it was observed that there was a significant decrease in the albumin and increase in the β-globulin resulting in significant increase in the total globulins and decrease in the A/G ratio. While α-1 component remained unchanged in all the repeatedly infected groups, α-2 increased only in the group infected with 500 + 500 larvae. The γ-globulin decreased in all the groups but remained unchanged in that infected with 500 + 500 larvae (Table). The decrease in the albumin and increase in β-globulin showed no correlation with the increasing doses of repeated infection ($p > 0.05$) suggesting that these changes were independent of repeated exposures of infection. The decrease in the albumin and increase in β-globulin was less pronounced in repeatedly infected groups as compared with those of single dose infected groups. When immunized groups which received 500 + 500 and 500 + 500 + 1000 larvae were compared with their non-immunized counterpart groups which received 500 and 1000 larvae, no significant alteration in albumin and β-globulin levels ($p > 0.05$) was seen, but when the heavily immunized group (250 + 500 + 1000 + 2000 + 4000 larvae) was compared with the non-immunized group (4000 larvae), a significant increase was seen in albumin ($p < 0.05$) but not in β-globulin ($p > 0.05$).

The present findings reveal that serum protein alterations were in accordance with the increasing single dose levels, but did not depend upon repeated dose levels. No differences occurred in serum protein of mice repeatedly infected with 500 + 500 and 500 + 500 + 1000 larvae when compared with their single dose counterparts. Animals which received a dose of 4000 larvae died, indicating their incompetence to exihibit any measurable immune response. Further investigations are being carried out on certain immunological aspects of *A. caninum* infection in mice, and the results will be available in due course[18].

Summary. Electrophoretic pattern of serum protein in mice during *Ancylostoma caninum* larval infection with various single and repeated doses were observed to acertain whether these changes take place according to their increase in dose levels. Comparison between single and repeatedly infected groups were also made statistically.

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The N-Terminal Amino Acid Sequence of the Small Subunit of Ribulose-1,5-diphosphate Carboxylase from *Nicotiana tabacum*

The sequence of the first 21 N-terminal amino acids of Fraction I protein small subunit from tobacco was determined, using a Beckman 890C automatic sequencer.

Ribulose-1,5-diphosphate carboxylase (Fraction I protein; E.C. 1.1.4.39) is the major soluble protein present in green leaves of plants which fix carbon via the Calvin (C_3) pathway. The protein has a molecular weight of about 525,000 and can be dissociated using a variety of techniques, including sodium dodecyl sulfate[1], urea[2], alkali[3] and phenol:acetic acid:urea (2:1:1 v/v/v)[4] into 2 types of subunits. The larger subunit has a molecular weight of 50–60,000 and the smaller a molecular weight of 12–25,000, the absolute molecular weight depending on the dissociating system used. Extensive work using peptide mapping techniques in the laboratory of S. G. Wildman has provided evidence for the inheritance of the 2 subunits being determined by 2 separate genetic systems: the small subunit exhibiting a classical Mendelian segregation in reciprocal crossing experiments whilst the large subunit appears to be controlled by factors inherited solely via the maternal side in a reciprocal cross[5–7].

In the genetic analysis of protein inheritance, knowledge of the amino acid sequence of the protein in question is always of great advantage. With the event of automatic protein sequencing equipment, this step of the work is greatly simplified. We present here the amino acid sequence of the first 21 amino acid residues from the N-terminal region of the small subunit of ribulose-1,5-diphosphate carboxylase from tobacco (*Nicotiana tabacum* var. Turkish Samsun).

Experimental. Ribulose-1,5-diphosphate carboxylase was prepared from leaves of 8-week-old glasshouse grown plants of *Nicotiana tabacum* var. Turkish Samsun by the direct crystallization method of CHAN et al.[8] with several modifications[4], the major change being that after Sephadex G-25 chromatography, the protein was purified by gel filtration on Sepharose 6B prior to crystallization – a

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5 10 15 20

Met Gln Val Trp Pro Pro $\begin{matrix}\text{Tyr}\\ \text{Ile}\end{matrix}$ Asn Lys Lys Lys Tyr Glu Thr Leu Ser Tyr Leu Pro Asp Leu

The N-terminal amino acid sequence of the small subunit of ribulose-1,5-diphosphate carboxylase from *Nicotiana tabacum*.