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.

M. K. BHOPALE and G. N. JOHRI

School of Studies in Zoology, Vikram University, Ujjain (India), 24 September 1974.

[18] We thank Professor H. SWARUP, Head, School of Studies in Zoology, Vikram University, Ujjain for providing laboratory facilities. These investigations were financed by funds from the Lady Tata Memorial Trust, Bombay.

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

[1] A. C. RUTNER and M. D. LANE, Biochem. biophys. Res. Commun. *28*, 531 (1967).
[2] K. E. MOON and E. O. P. THOMPSON, Aust. J. biol. Sci. *22*, 463 (1969).
[3] N. KAWASHIMA and S. G. WILDMAN, Biochem. biophys. Res. Commun. *41*, 1463 (1970).
[4] G. C. GIBBONS and S. STRØBAEK, in preparation.
[5] P. H. CHAN, Ph. D. thesis, University of California, Los Angeles (1972).
[6] P. H. CHAN and S. G. WILDMAN, Biochim. biophys. Acta *277*, 677 (1972).
[7] N. KAWASHIMA and S. G. WILDMAN, Biochim. biophys. Acta *262*, 42 (1972).
[8] P. H. CHAN, K. SAKANO, S. SINGH and S. G. WILDMAN, Science *176*, 1145 (1972).

				5					10					15					20	
Met	Gln	Val	Trp	Pro	Pro	Tyr / Ile	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*.

step which enhanced the crystallization by first removing aggregated material and lower molecular weight proteins, and secondly shortening the time of crystallization.

The small subunit of ribulose-1,5-diphosphate carboxylase was prepared by reduction of 3 times crystallized native protein with 100 m*M* β-mercaptoethanol in the presence of 50 m*M* sodium phosphate buffer pH 11.2 for 30 min and subsequent separation of the large and small subunits by gel filtration on a Sephadex G-100 column equilibrated in 50 m*M* phosphate buffer pH 11.2. Fractions containing the small subunit, which eluted with a K_{av} value of 0.38 (corresponding to a molecular weight of 25,000) were pooled and the buffer exchanged to 0.2 *M* ammonium carbonate (pH 8.9) by Sephadex G-25 chromatography. The small subunit was then lyophilized to a salt-free white hygroscopic powder. Samples (5 mg) of this material were either used directly for sequence determination, or S-carboxymethylated and then sequenced.

The N-terminal amino acid sequence of the small subunit was determined by automatic Edman degradation on a Beckman sequencer, Model 890C. The reagents, solvent systems and methods for identification of phenylthiohydantoin amino acids were as described by Scawen and Boulter[9].

Results and discussion. The N-terminal amino acid sequence of the first 21 residues of the small subunit of tobacco ribulose-1,5-diphosphate carboxylase is presented in the Figure. The methionine residue at the N-terminus was found in variable yields. When the N-terminal Met yield was low, Gln 2 was also released in cycle 1 and in subsequent cycles a phase-shifted minor amino acid was observed, i.e. cycle 2, Val + Gln; cycle 3, Trp + Val; cycle 4, Pro + Trp, etc. The variable yield of N-terminal Met may be due to partial removal of the amino acid during isolation and purification of the small subunit or due to an incomplete removal in vivo in a similar way as in prokaryotes. On present evidence, however, all plant proteins should start with Met[10].

The polymorphism at residue 7, Tyr/Ile, was observed in all runs, and the two amino acids occurred in equal amounts. This Tyr/Ile pair was also found in cycle 6 under conditions where the yield of Met 1 was low. The polymorphism seen here, in the light of the wide variation in amino acid composition of the small subunit observed in *Nicotiana* spp.[11], may well be due to the fact that the small subunit is a rapidly evolving peptide.

The ability to determine the N-terminal sequence of the small subunit of ribulose-1,5-diphosphate carboxylase from tobacco by automatic Edman degradation is in itself noteworthy. Repeated attempts in the laboratory to determine the N-terminus by classical manual Edman degradation followed by dansylation, have been unsuccessful. This inability to determine the N-terminal amino acid of the small subunit of ribulose-1,5-diphosphate carboxylase has also been reported by Moon and Thompson[12] in the case of the spinach beet enzyme, and by Iwanij et al.[13] for *Chlamydomonas reinhardi* where the authors suggested that the N-terminus was blocked.

Summary. The N-terminal sequence of the small subunit of Fraction I protein isolated from tobacco was investigated, using an automatic protein sequencer. The amino acid sequence of the first 21 residues is presented[14].

G. C. Gibbons[15], S. Strøbaek[16]
B. Haslett[17] and D. Boulter[17]

Carlsberg Research Department, Strandvejen 50, DK-2900 Hellerup (Denmark), Institute of Genetics, University of Copenhagen, Øster Farimagsgade 2A, DK-1353 Copenhagen K (Denmark), and Department of Botany, University of Durham, Durham City DH1 3LE (England), 26 May 1975.

[9] M. D. Scawen and D. Boulter, Biochem. J. *143*, 257 (1974).
[10] A. Yarwood, D. Boulter and J. N. Yarwood, Biochem. biophys. Res. Commun. *44*, 353 (1971).
[11] S. Y. Kwok, Ph. D. thesis, University of California, Los Angeles (1972).
[12] K. E. Moon and E. O. P. Thompson, Aust. J. biol. Sci. *24*, 755 (1971).
[13] V. Iwanij, N.-H. Chua and P. Siekevitz, Biochim. biopys. Acta *358*, 329 (1974).
[14] Acknowledgments. The work was supported in part by grants from The Carlsberg Foundation and USPHS-NIH grant No. GM-10819. G.C.G. and S.S. thank Professors B. Foltmann and D. von Wettstein in whose laboratories the enzyme preparation was carried out.
[15] Carlsberg Research Department, Strandvejen 50, DK-2900 Hellerup, Denmark.
[16] Institute of Genetics, University of Copenhagen Øster Farimagsgade 2A, DK-1353 Copenhagen K., Denmark.
[17] Department of Botany, University of Durham, Durham City DH1 3LE, U.K.

Die Schizogonie bei *Eimeria tenella* (Sporozoa, Coccidia)

Schizogony in *Eimeria tenella* (Sporozoa, Coccidia)

Bei der klassischen Schizogonie der Sporozoen ist über den Ablauf der Kernteilungen und über die Bildung der Tochterzellen noch wenig bekannt[1-6].

In Untersuchungen am Hühnercoccid *Eimeria tenella* wird die Feinstruktur der zweiten und dritten Schizontengeneration in ihrer fortschreitenden Entwicklung dargestellt[7]; der Vorgang, der dieser Massenvermehrung zugrunde liegt, wird bei *Eimeria tenella* im elektronenmikroskopischen Bild belegt. Die Schizogonie beginnt hier mit der Umwandlung von Merozoiten in intrazelluläre einkernige Wachstumsstadien in der Blinddarmmucosa und -submucosa.

Zwei Entwicklungsschritte folgen bei der Schizogonie aufeinander: die Kernvermehrung und die anschliessende Tochterzellbildung (=Merozoitenbildung). Bei jeder Kernteilung bildet sich ein exzentrisch gelegener intranukleärer Spindelapparat, Centriolen sind den Spindelpolen im Cytoplasma vorgelagert. Vor einer Kernteilung teilt sich jeweils das zum Kern gehörige Dictyosom. Ähnlich wie bei *Eimeria necatrix*[1] und *Eimeria ninakohly-*

[1] J. F. Dubremetz, Ultrastruct. Res. *42*, 354 (1973).
[2] J. F. Dubremetz, J. Protozool. *22*, 71 (1975).
[3] D. M. Hammond, W. L. Roberts, N. N. Youssef and H. D. Danforth, J. Parasit. *59*, 581 (1973).
[4] G. Heller und E. Scholtyseck, Protistologica *7*, 451 (1971).
[5] E. S. Snigirevskaya, Acta protozool. *7*, 57 (1969).
[6] B. Chobotar, E. Scholtyseck, J. Senaud and J. V. Ernst, Z. Parasitenk. *45*, 291 (1975).
[7] G. Hoppe, Diss. Univ. Bonn (1973).