

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 mM β -mercaptoethanol in the presence of 50 mM 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 mM 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 BOULTER⁹.

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 Met¹⁰.

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 THOMPSON¹² in the case of the spinach beet enzyme, and by IWANIJ et al.¹³ for *Chlamydomonas reinhardtii* 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 presented¹⁴.

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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. biophys. Acta* **358**, 329 (1974).

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

Schizogonie 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 bekannt¹⁻⁶.

In Untersuchungen am Hühnercoccid *Eimeria tenella* wird die Feinstruktur der zweiten und dritten Schizontengeneration in ihrer fortschreitenden Entwicklung dargestellt⁷; 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 ninakholy-*

¹ J. F. DUBREMETZ, *Ultrastruct. Res.* **42**, 354 (1973).

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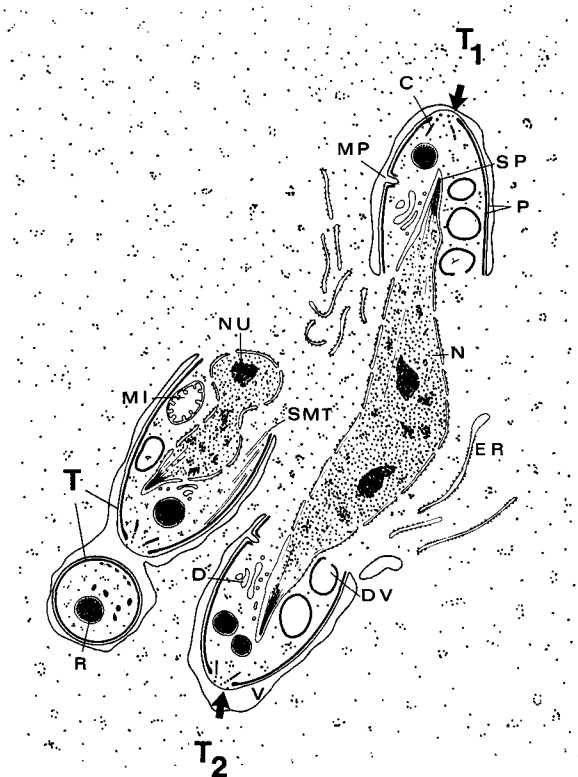
⁴ G. HELLER und E. SCHOLTYSECK, *Protistologica* **7**, 451 (1971).

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⁶ B. CHOBOTAR, E. SCHOLTYSECK, J. SENAUD and J. V. ERNST, *Z. Parasitenk.* **45**, 291 (1975).

⁷ G. HOPPE, *Diss. Univ. Bonn* (1973).

*akimovae*⁸ kann bei *Eimeria tenella* eine Kernteilung einsetzen, bevor die vorangegangene abgeschlossen ist. Ein genauerer Bericht über Wachstum und Kernteilungsvorgänge im Schizonten folgt an anderer Stelle.



Schematische Darstellung von 4 Tochterzellen (= Merozoiten) in der Randzone des Schizonten. T₁, T₂ zu einem Drittel differenzierte, synchron heranwachsende Tochterzellen (T) über den Spindelpolen (SP) eines Schizontenkerns (N); C, Conoid; DV, dickwandiger Vesikel; ER, endoplasmatisches Reticulum; MI, Mitochondrium; MP, Mikropore; NU, Nukleolus; P, Pellicula; R, Rhoptrienanlage; SMT, subpelliculärer Mikrotubulus; V, Vakuole in Verbindung mit der parastophoren Vakuole.

Die Bildung von Tochterzellen ist mit der letzten Teilung der Schizontenkerne in der Mutterzelle gekoppelt und unterscheidet sich nicht wesentlich von den früheren Kernteilungen. In einer neueren Arbeit wird die Tochterzellbildung dargestellt⁹. Über jedem Kernspindelpol entsteht die Anlage einer Tochterzelle. Sie hat ein Conoid und einen inneren Membrankomplex, der nach aussen von einer Cytoplasmamembran kappenartig begrenzt wird. Zu diesem frühen Zeitpunkt der Entwicklung lassen sich auch 4 Tochterzellanlagen an zwei noch nicht vollständig getrennten Kernen beobachten. Die Pelliculamembranen der Tochterzellanlagen vergrössern sich synchron während der fortschreitenden Teilung des Schizontenkerns und umschliessen dabei einen Spindelpol mit den benachbarten Centriolen und ein Dictyosom (Figur).

Die neuen Ergebnisse zeigen feinstrukturelle Übereinstimmungen im Vermehrungsablauf bei *Eimeria tenella* und den Toxoplasma (Biocca 1968) und sprechen für eine enge verwandtschaftliche Zusammengehörigkeit.

Summary. Fine structural aspects of development in 2nd and 3rd generation schizonts were studied with experimentally infected chickens. Two developmental steps were observed in schizogony: multiplication of nuclei and succeeding merozoite formation associated with the last division of each nucleus in the schizont. Dividing nuclei had eccentric intranuclear spindles and centrioles near their poles.

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⁸ G. L. KELLEY and D. M. HAMMOND, J. Parasit. 59, 1071 (1973).

⁹ G. HOPPE, Protistologica 10, 185 (1974).

Labelling of TCA-Soluble Fraction and Protein of the Rat Visual Cortex After Learning a Pattern Discrimination Task

The possibility of training to perform a visual vertical-horizontal discrimination monocularly has been demonstrated by BUREŠOVÁ and NADEL¹. Intact visual cortex contralateral to the intact eye was shown to be essential for acquisition and retention of the new experience. No transfer to the opposite hemicortex takes place. This paradigm was used in the present experiments in an attempt to find out whether this kind of learning would cause any difference in the labelling of protein in the visual hemicortex of rat.

Materials and methods. Experiments were performed on the male hooded rats weighing 180–200 g. The horizontal-vertical discrimination training procedure was essentially that already published¹. Briefly, visual input was restricted by covering one eye with an opaque plastic occluder. The rats were trained to escape or avoid shocks during 5 sec by entering an unlocked door. Immediately after reaching a criterion of 12 correct in 13 consecutive responses, the occluder was removed.

After 30 min of rest (period of maximal chemical response²), 0.5 ml saline containing about 16 μ Ci L-(U-¹⁴C)-leucine or about 13 μ Ci L-(1-¹⁴C)-leucine was injected s.c. After definite time intervals, the animals were sacrificed by decapitation in a cold room at 2–4°C. The samples of both visual hemicortices were dissected with the aid of a celluloid stencil³ and frozen in liquid nitrogen. Tissue samples were then homogenized in the ice-chilled 6% trichloroacetic acid (TCA) containing 2 mg/ml ¹²C-leucine. TCA-precipitates were dissolved in 1 N NaOH after treatment by the procedure⁴. In aliquots,

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⁴ P. SIEKEVITZ, J. biol. Chem. 195, 549 (1952).