We found we could use this procedure to follow the decline of rat serum vitamin A during the onset of vitamin A deficiency. Male weanling rats placed on an A deficient diet experienced a fall in average serum retinol from 66 µg/100 ml to 12 µg/100 ml over a 52-day period. This method was useful for weanling rats since less than a ml of blood was needed for each assay.

The cross reactivity of the antiserum with various retinoids is relevant to the applicability of this RIA to retinol in fasting serum. The following facts indicate that the use of the antiserum for this purpose is appropriate: only a tiny fraction of the retinoids in the body are present as retinoic acid[5]; retinal is formed in the tissue where it is used[5]; retinyl esters and carotenes are prominant in serum only after eating[6], and in any case, react very poorly with the antibody[2].

The values obtained in this work for the retinol levels of normal rat serum extend from 46 to 57 µg/100 ml (table 2) and are consistant with published values[7,8]. Suthutuoravoot and Olson[9] have pointed out that normal serum vitamin A concentrations can vary widely and reflect not only vitamin A nutrition but also protein intake.

The RIA described here detects as little as 1 ng of retinol per assay tube. High performance liquid chromatography (HPLC) techniques provide the same order of sensitivity; they are reported to detect as little as 5–7 ng of vitamin A[10,11]. HPLC has the added virtue that it will separate the various retinoids as well as quantitate them. The RIA reported here has an advantage over HPLC for the routine measurement of serum retinol; sample preparation is simpler since it consists only of ethanol addition and centrifugation while the HPLC techniques require solvent extraction, and drying down or lyophilization[10,11]. Furthermore, the RIA technique can be applied in any laboratory to which a scintillation counter is available.

1 This work was supported by United States Public Health Service grant 2 RO1 AM19716.
2 D.H. Conrad and G.H. Wirtz, Immunochemistry *10*, 272 (1973).
3 G.E. Abraham, J.E. Buster and R.C. Teller, Analyt. Lett. *5*, 757 (1972).
4 D. Rodbard, Clin. Chem. *20*, 1255 (1974).
5 D.S. Goodman, Fedn Proc. *38*, 2501 (1979).
6 D.S. Goodman, in: Vitamins and Hormones, vol. 32, p. 167. Ed. R.S. Harris, P.L. Munson, E. Diczfalusy and J. Glover. Academic Press, New York 1974.
7 J.B. Neeld and W.N. Pearson, J. Nutr. *79*, 454 (1963).
8 H.E. Sauberlich, R.E. Hodges, D.L. Wallace, H. Kolder, J.E. Canham, J. Hood, N. Raica, Jr. and L.K. Lowry, in: Vitamins and Hormones, vol. 32, p. 251. Ed. R.S. Harris, P.L. Munson, E. Diczfalusy and J. Glover. Academic Press, New York 1974.
9 S. Suthutuoravoot and J.A. Olson, Am. J. clin. Nutr. *27*, 883 (1974).
10 C.A. Frolik, T.E. Tavela and M.B. Sporn, J. Lipid Res. *19*, 32 (1978).
11 M.G.M. DeRuyter and A.P. De Leenheer, Clin. Chem. *24*, 1920 (1978).

A blocked mutant of *Claviceps purpurea* accumulating chanoclavine-I-aldehyde[1]

W. Maier, D. Erge, J. Schmidt and D. Gröger

Institute of Plant Biochemistry, Academy of Sciences of the German Democratic Republic, DDR-4010 Halle/Saale, Weinberg (German Democratic Republic), 13 March 1980

Summary. An alkaloid-blocked mutant of *Claviceps purpurea* was isolated from a strain which produces ergotoxine alkaloids.

The mutant accumulates chanoclavine-I and the corresponding aldehyde. It lacks the ability to form tetracyclic ergolines.

Blocked mutants are particularly useful for studying the genetics of microorganisms producing secondary metabolites and the biosynthetic pathways of natural products. Furthermore, new compounds may be obtained by 'mutational biosynthesis'. This technique has been widely applied in the field of antibiotics[2–4].

Apparently alkaloid-blocked mutants have not yet been described. We wish to report our results with *Claviceps purpurea* (Fr.) Tul.

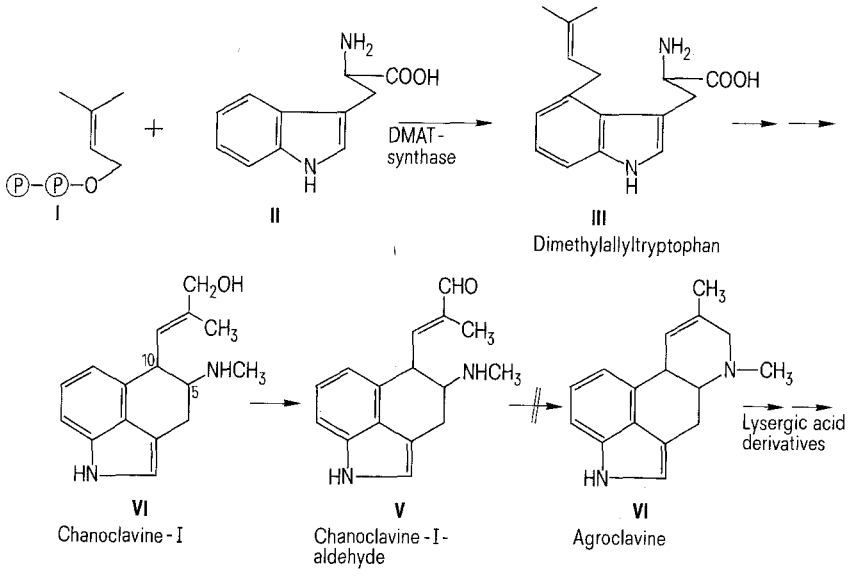
The parent strain *Claviceps purpurea* Pepty 695/S, in a sucrose-ammonium citrate medium[5] under submerged conditions, produces 1.2 g/l alkaloids. The alkaloid mixture is composed of ergotoxine (60%), ergometrine (20%), and clavine alkaloids including chanoclavine-I (20%).

During a screening program we selected single colonies and tested their ability to form alkaloids in a production medium[5]. Surprisingly 1 isolate, which showed no changes in morphology and pigmentation compared with the parent strain, did not synthesize any lysergic acid derivatives. The total alkaloid yield of this strain, designated Pepty 695/ch, amounted to 0.5 g/l. TCL (silica gel PF_{254} Merck) of a crude alkaloid extract revealed 2 spots designated A and B. R_f values of A and B in comparison to agroclavine and elymoclavine in different developing systems were as follows. In chloroform/methanol (80:20, v/v): A 0.08; elymoclavine 0.38; B 0.44; agroclavine 0.6. In methanol: A 0.12; B 0.27; agroclavine and elymoclavine 0.44. In chloroform/tert. butanol (3:1, v/v) 15% ammonia atmosphere: A 0.39; elymoclavine 0.48; agroclavine 0.88. 1 g of a crude alkaloid mixture obtained according to Erge et al.[5] and Maier et al.[6] was separated by repeated preparative TLC. Compound A was crystallized from acetone and B was obtained as an amorphous substance. The proportion of A: B was 4:1.

A was identified as chanoclavine-I (**IV**) and B as chanoclavine-I-aldehyde (**V**) on the basis of the following data.

A: m.p. 215 °C; $[\alpha]_{20}^{D} = -235°$ (c 1.0 in pyridine). $C_{16}H_{20}N_2O$[by HRMS, M^+ m/e 256,1586 (M^+ calc. m/e 256, 1576)]. Fragmentation: m/e 237; 223; 196; 183 ($C_{12}H_{11}N_2$, found 183, 0935, calc. 183.0922) 168, 167, 155, 154. PMR (100 MHz, CD_3OD, values are in ppm, δ scale): 1.85 (vinyl-CH_3, weak d); 2.42 (N-CH_3, s); 3.85–3.95 (C-10-H, m), 4,13 (CH_2O, br. s); 5.35 (vinyl-H, dq).

B: $C_{16}H_{18}N_2O$ [by HRMS, M^+ m/e 254,1414 (M^+ calc. m/e 254, 1419)]. Fragmentation: m/e 237 ($C_{16}H_{17}N_2$, found 237,1407, calc. 237, 1392), 235, 223, 211, 194 ($C_{14}H_{12}N$, found 194,1017, calc. 194,097), 183, 168, 167 155, 154. After treatment with D_2O a peak at m/e 256 was recorded. PMR (100 MHz, $CDCl_3$) 1.95 (vinyl-CH_3, weak d); 2.52 (N-CH_3, s); 4.33 (C-10-H, m) 8.0 (indol-NH, s), 9.4 (-CHO, s). IR



Biosynthetic pathway of ergoline formation. Bars indicate position of block in strain Pepty 695/ch.

showed a α,β-unsaturated aldehyde band (1685 cm^{-1}). Reduction of B with $NaBH_4$ gave chanoclavine-I.

This is the first report of the occurrence of chanoclavine-I-aldehyde in ergot. Synthetically prepared **V** is efficiently and specifically converted into tetracyclic ergolines[7,8].

In addition to Naidoo et al.[7] and Floss et al.[8] our results support the view, that chanoclavine-I-aldehyde is indeed a natural intermediate in ergoline alkaloid biosynthesis (figure).

We compared the activities of both the dimethylallylpyrophosphate: tryptophan dimethylallyl transferase (DMAT-synthase) and chanoclavine-I-cyclase[9] in the parent strain and in the mutant at the beginning of the idiophase. Both strains showed the same DMAT-synthase[10] activity. But there was a drastically reduced activity of chanoclavine-I-cyclase, which catalyzes the conversion of **IV**→**VI**, in strain Pepty 695/ch. The parent strain (Pepty 695/S) showed a conversion rate of 45% of the substrate into agroclavine, whilst the mutant gave a conversion of 2–3% into chanoclavine-I-aldehyde and 5% into agroclavine.

A reason why tetracyclic ergolines are not synthesized in strain Pepty 695/ch in vivo is obviously the blocking of the chanoclavine-I-cyclase activity under these conditions. However, it is surprising that crude enzyme extracts of the mutant are able to form at least small amounts of agroclavine from **IV**. **V** was observed as reaction product of chanoclavine-I-cyclase only with the mutant but never with other *Claviceps* strains.

1 Dedicated to Prof. Dr Dr h.c. K. Mothes on the occasion of his eightieth birthday.
2 S.W. Queener, O.K. Sebek and C. Vézina, A. Rev. Microbiol. *32*, 593 (1978).
3 K. Nagaoka and A.L. Demain, J. Antibiot. *28*, 627 (1975).
4 S.J. Daum and J.R. Lemke, A. Rev. Microbiol. *33*, 241 (1979).
5 D. Erge, A. Wenzel and D. Gröger, Biochem. Physiol. Pfl. *163*, 288 (1972).
6 W. Maier, D. Erge and D. Gröger, Biochem. Physiol. Pfl. *161*, 559 (1971).
7 B. Naidoo, J.M. Cassady, G.E. Blair and H.G. Floss, Chem. Commun. *1970*, 471.
8 H.G. Floss, M. Tscheng-Lin, Ch.-J. Chang, B. Naidoo, G.E. Blair, Ch.I. Abou-Chaar and J.M. Cassady, J. Am. chem. Soc. *96*, 1898 (1974).
9 D. Erge, W. Maier and D. Gröger, Biochem. Physiol. Pfl. *164*, 234 (1973).
10 W. Maier and D. Gröger, Biochem. Physiol. Pfl. *170*, 9 (1976).

The use of a Quantimet image analysis system to analyse trypsin banding patterns of V79/4 (AH1) Chinese hamster chromosomes

C.J. Roberts and J.N. Pritchard

Environmental and Medical Sciences Division, A.E.R.E., Harwell OX11 ORA (England), 22 November 1979

Summary. A Quantimet image-analysis system was programmed to identify and print out banding patterns from G-banded chromosome spreads of a clone derived from the established cell line V79/4 (Chinese hamster fibroblasts), designated V79/4(AH1).

The use of chromosome banding patterns has made identification of individual chromosomes and their abnormalities a more positive and clear procedure than was formerly the case. While, for obvious reasons, much of this work has been directed to the study of human chromosomes, other mammalian species have also received considerable attention. Among these is the Chinese hamster, cultured cells of this species have been widely used in cytogenetics and radiobiology because of their low chromosome number ($2n=22$) and stability in culture[2,3].

The aim of our research program is to use banding techniques to investigate whether or not a visible structural