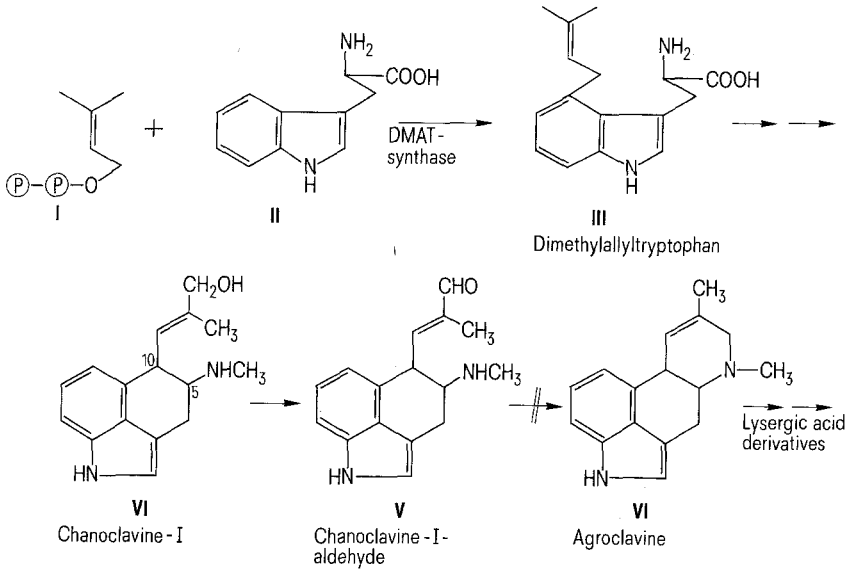

Biosynthetic pathway of ergoline formation. Bars indicate position of block in strain Pepty 695/ch.

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

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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

chromosome aberration in the X chromosome is regularly associated with drug-resistant variants induced in a subline of the Chinese hamster lung fibroblast cell line V79/4, after exposure to various ionising radiations[4]. We are particularly interested in those variations involving the hypoxanthine-guanine phosphoribosyl transferase (HGPRT 2.4.2.8.) locus located on the p arm of the X chromosome.

We have developed a method of using a Quantimet 720 image-analysis system (Cambridge Scientific Instruments Ltd, Cambridge, GB) to identify and print out G-banding patterns. Before it can be applied to the study of mutants, it is necessary to establish the G-banding pattern of our cloned subline of V79/4, designated V79/4(AH1), and we here report this G-banding pattern.

The V79/4(AH1) Chinese hamster lung fibroblasts were grown as a monolayer in MEM, on Earles salts supplemented with 10% foetal bovine serum and 7.5×10^{-4} M glutamine. Supplemented medium was equilibrated with 5% CO_2 in air. Cultures were harvested after 48 h, by trypsinization after 3 h colcemid treatment (0.8 µg/ml), final conc. Cells were treated with hypotonic solution containing 0.5% trisodium citrate and 0.28% potassium chloride dissolved in double distilled water (DDW), for 10 min at 36 °C. Cells were then washed and fixed in 3 changes of ethanol:glacial acetic acid (3:1), then air dried on to pre-cleaned glass slides. 7 days later chromosome preparations were treated for 35–45 sec with 0.25% trypsin made up in phosphate buffered saline (PBS)[5], the slides were then rinsed in PBS. Stock Giemsa (R.A. Lamb), was diluted (1:9) with DDW, and was then twice filtered prior to use; slides were stained for 5 min then washed in DDW, air dried and mounted in DPX (R.A. Lamb).

After mounting, slides were photographed using a Nikon Optiphot photomicrography system (Projectina Co.) with a ×100 oil immersion lense. The photographic negatives were enlarged 4 times to give a final magnification of ×4000. The photomicrographs were then mounted on an epidiascope from which they were scanned by a Plumbicon television camera. The image was split into 600,000 points, each point having an associated optical density expressed as one of 64 discrete grey-levels on an approximately linear scale. The image was displayed on a screen where individual chromosomes were selected for measurement using a light-pen editing facility. Control of the system hereafter is software based, in this case by a desk-top computer (Hewlett-Packard Ltd, model No.9825A). A more detailed description of the Quantimet system and its capabilities has been given elsewhere[6].

The computer steps a measurement frame of predetermined proportions across the chromosome, measuring the average grey-level within each frame. This information is then used to print an image of the chromosome using a set of characters, each character representing a particular range

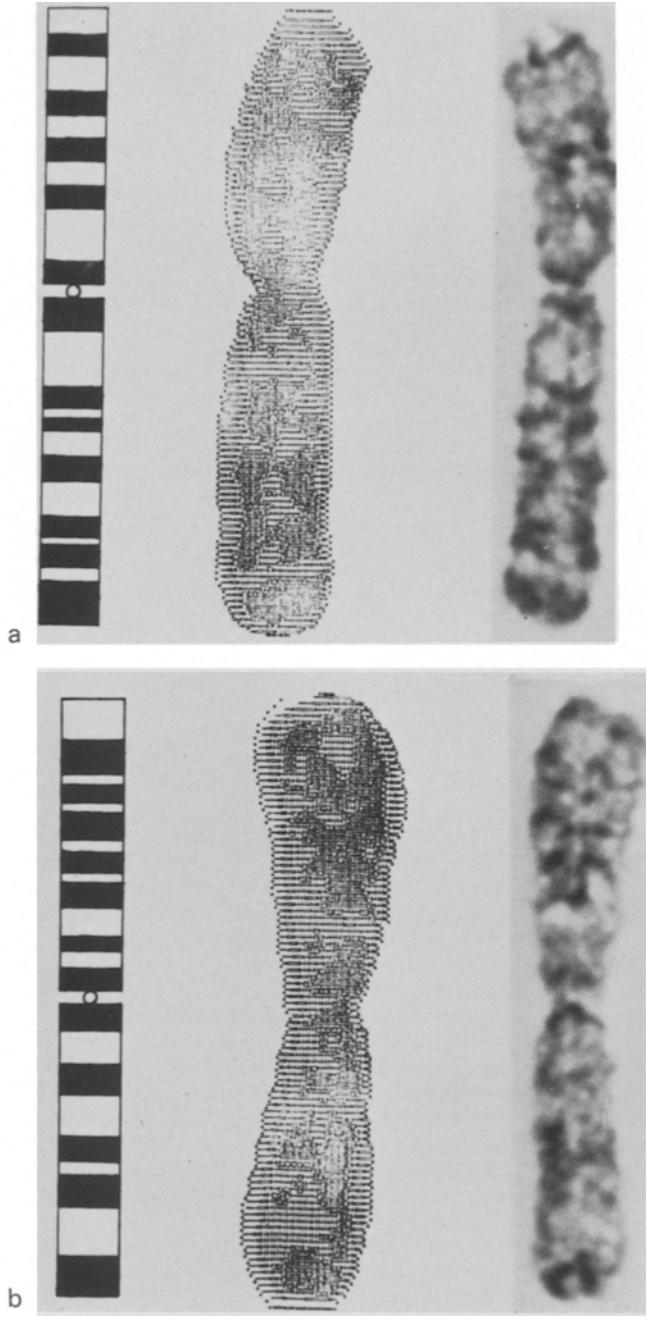

Fig. 1, a and b. From the photomicrograph on the left of the figures the image analysis system produces a graphic output shown in the centre of figure 1, a and b. From this output the number and position of the bands can be estimated as shown in the 3rd diagram in each figure.

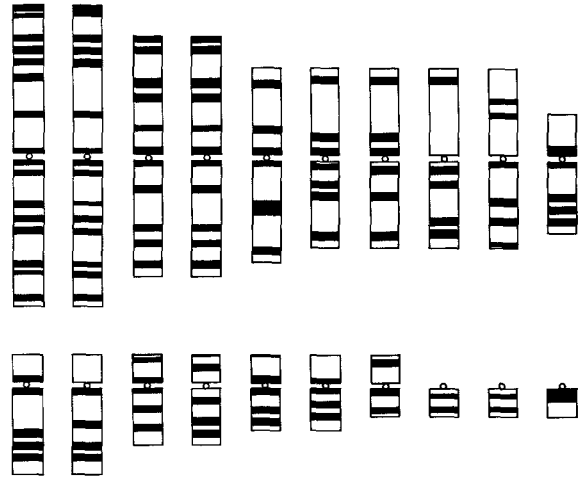

Fig. 2. G-banded karyotype of V79/4(H1) was constructed by using the graphics generated by the image analysis system using suitable chromosome preparations and assembled as detailed in the text and in figure 1, a and b. Chromosome 2 although sub-metacentric has a similar banding pattern to chromosome 1, and is shown in this diagram as being metacentric.

of optical densities. These optical density ranges can be readily manipulated to enable the boundary of the bands within a chromosome to be well defined. The printer (Hewlett-Packard Ltd, model No.9871A) is automatically programmed for character spacing in order to prevent distortion, the result of which is a correctly proportioned, permanent record of the chromosome banding pattern.

The time taken for analysis depends on the resolution required, but an average time is about 10 min per chromosome. If required, data may be transferred to a central computer for further numerical analysis or storage on magnetic tape.

Counting of 100 spreads was done to establish the modal chromosome number of V79/4(AH1), which was found to be 20. The diploid chromosome number of Chinese hamster is 22.

A photomicrograph of a typical hamster chromosome is shown in figure 1, together with the graphical output from the Quantimet and a diagram to clarify and demonstrate the method by which we obtain band numbers on the individual chromosomes. Figure 2 was constructed using Quantimet generated graphics of three V79/4(AH1) cell spreads. This data were combined to give the banding patterns shown and it can be seen from this figure that V79/4(AH1) does not display 9 regular autosomal pairs plus the sex chromosomes X, Y. Instead, we have arranged all the chromosomes in descending order of size. Some degree of band homology can be seen in chromosomes 1,2 and 3,4 but the rest are difficult to pair. The sex chromosomes of V79 were identified using C-banding (unpublished data), the X being No. 9.

The use of an image analysis system has made the skilled and difficult job of chromosome identification a simpler and more reliable operation. The process has also allowed us to identify very faint bands which are extremely difficult to detect with the naked eye. With this system is also possible to store information from the photomicrographs for later retrieval or for comparisons to be made between photomicrographs.

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Detection of nucleolus organizer regions (NOR) in the chromosomes of the domestic pig (*Sus scrofa domestica* L.)

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Summary. In the domestic pig (*Sus scrofa domestica* L.) nucleolar organizer regions (NOR) were detected by a combined silver-Giemsa method (Ag-G). The main sites of NORs are the secondary constrictions of chromosomes 8 and 10. Sometimes an additional NOR was observed near the centromere of 1 homologue of chromosome 11. Associations of NORs were seen only between chromosomes 10 and at a very low frequency.

In man, the position of the ribosomal cistrons coding for 18S and 28S RNA on the secondary constrictions of the short arms of the 5 acrocentric chromosomes was originally determined by hybridization studies in situ[1,2], and these nucleolar organizer regions (NORs) have been confirmed in many cases with the use of silver staining techniques[3,4]. It could be demonstrated that only those NORs which were functionally active during the preceeding interphase are stainable with silver in mitotic cells[5,6]. The material which stains with silver positively is not the ribosomal DNA itself but an acidic protein associated with the rRNA transcribed at the ribosomal DNA sites[3,7]. It is well documented, especially for human materials, that the patterns of expression and size of silver stained NORs are characteristic for each individual, typical for the species, and heritable[3,8].

In the pig the Ag-AS technique was applied for the differential staining of NORs[9]. Sysa et al.[10] used an N-band technique for this purpose. They reported that the N-bands were clearly located in the centromeres and in the secondary constrictions of several chromosomes. The exact identification of the NOR-bearing chromosomes has not yet been done on the basis of banding patterns. This study demonstrates the silver staining patterns in the chromosomes of the domestic pig using a combined silver-Giemsa technique[11].

Materials and methods. Chromosomes of 10 domestic boars (4 Pietrain, 2 Belgian Landrace, 2 Österr. Landrasse and 2 Deutsches Edelschwein, from artificial insemination stations in upper and lower Austria) were prepared from phytohaemagglutinin-stimulated lymphocytes following short term cultures. We only took male pigs because, in contrast to female pigs, the breeding conditions were exactly known. Slides were stained using a combined Ag-Giemsa technique for demonstrating both NORs and G-bands[11]. Chromosome preparations were first stained with a modified silver method according to the staining of Mikelsaar et al.[12]. For G-banding a pretreatment in phosphate buffer (pH 6.8) for 10 min was necessary. Thereafter the preparations were trypsinized in 0.025% Trypsin (Difco) diluted in phosphate-buffer at pH 6.8 for 5–7 min. Then the slides were rinsed in distilled water and stained in phosphate-buffered (pH 6.8) Giemsa (Merck) 1:15 for 8 min at room temperature. After rinsing in distilled water the slides were dried and mounted in DPX. Chromosome identification followed the system of Gustavsson[13].

Results and discussion. In the domestic pig (*Sus scrofa domestica* L.; 2n = 38) the presence of secondary constrictions is variable. While chromosome pair 10 is always morphologically distinguishable by an evident secondary constriction, in chromosome 8 this region can be seen only in favourable cells. In a significant proportion of all metaphases examined the main sites of intensely stained NORs were confined to these regions. However, in a small number of cells an additional NOR may occur near the cen-