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 The sex chromosomes of V79 were identified using C-banding (unpublished data), the X being No. 9.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.

1 Acknowledgments. We thank Dr J.R.K. Savage and Dr J. Thacker, MRC Harwell for their advice, also Dr P.D. Holt for useful discussions. We also thank D. Coates for his assistance with the Quantimet. This work was partly supported by Euratom grant No. 134-74-1 BIOUK.

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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-

tromere in one of the homologues of chromosomes 11. A representative selection of silver staining patterns is illustrated in figure 1.

The quantity of silver precipitated was variable, with some chromosomes having very little and others having larger amounts (figure 2). Evans et al.[1] found the same with ^{3}H-rRNA hybridization to rDNA, indicating a variable number of genes coding for rRNA per NOR. This could be one reason for the variation on the amount of silver precipitated.

Variation was not only observed in the amount of the silver deposits but also in the number of stained NORs. The presence or absence of stain on characteristic chromosomes which varied little between the cells, produced a relatively constant pattern typical for each individual. Beyond that, a significant differences in mean numbers of NORs existed between the 4 races (an extensive study on the variation of NORs in the 4 races is in preparation). This might reflect the absence of rRNA sites or the absence of gene activity. These results agree with the findings reported for man and various mammals[3,14,15]. Here the question arises how many sites of rRNA genes exist at all in a chromosome complement, and which factors suppress genes coding for rRNA.

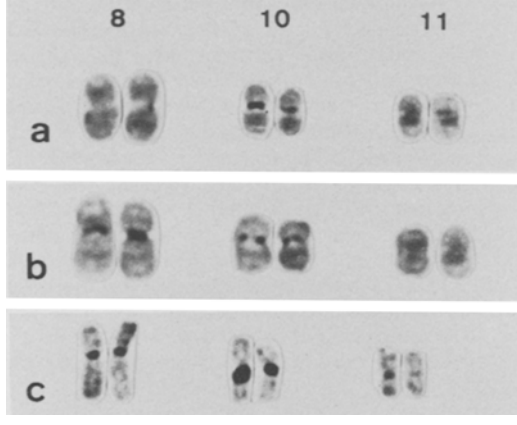

Fig. 1. Alternative Ag-Giemsa staining patterns of 3 individuals of the domestic pig (*Sus scrofa domestica* L.). a) Silver stained NORs only on chromosome pair 10. b) Silver stained NORs on chromosome pairs 8 and 10. c) Silver stained NORs on chromosome pairs 8 and 10 and one homologue of chromosome 11. Note the variation in amount of silver deposits.

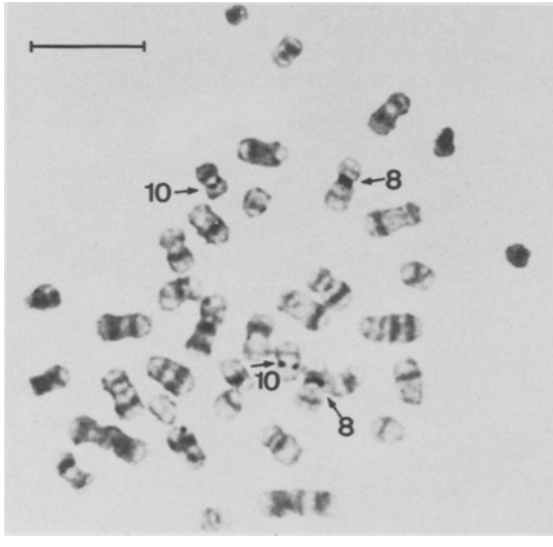

Fig. 2. Ag-Giemsa stained metaphase of a male pig. Arrows indicate the NORs on chromosomes 8 and 10. Scale 10 µm.

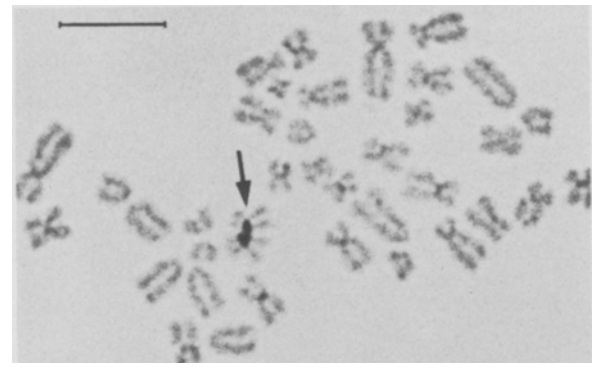

Fig. 3. Silver stained metaphase of a male pig. Arrow indicates the association complex between the chromosomes 10. Scale 10 µm.

Besides the main sites of silver stained NORs at the secondary constrictions and the region near the centromere, silver staining very often occurred also as minute dots at the centromeres of all chromosomes. Similar results concerning the simultaneous staining of NORs and kinetochores have been reported by several authors[16–18]. It seems possible that the silver stained centromeres also include material similar to that which in NORs stains positively. While in man and most of the animals investigated the forming of associations between NOR bearing chromosomes can be observed at a high frequency, in the pig in only 0.83% of all metaphases investigated could associations be found. In all cases the associations were restricted to NORs localized at the secondary constrictions of chromosomes 10 (figure 3). The location of NORs seems to be significantly correlated with the ability to form NOR-associations and even their frequency. NORs which are localized in the middle of the chromosomes seem to be less suited to forming association complexes than NORs in terminal positions.

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