

regions is demonstrated by the uniform staining of chromosomes with AO or by the Feulgen reaction. Furthermore, the assumed differential staining of DNA with

Giemsa as the basic mechanism which underlies the demonstration of constitutive heterochromatin is excluded by the staining of the latter in alkali-treated chromosomes extracted with DNase. However, the necessity of a longer staining after DNA extraction would suggest a stronger affinity of the Giemsa stain for the DNA-protein complex.

The high protein content of metaphase chromosomes¹⁷ makes it possible to explain the staining of constitutive heterochromatin with Giemsa (C bands, 7) in terms of an unequal loss of proteins along the chromosome or changes in their staining reactivity. In effect, the protein components of the arms could be more easily extractable by heat or alkaline treatments than those of the centromeric regions where satellite DNA is located^{1,19}, since it has been suggested¹⁸ that the latter is more firmly bound to chromosomal proteins. The fact that aldehyde postfixation, which binds chromosomal components in close association, allows DNA denaturation¹⁵ but prevents Giemsa differentiation after heat or alkali, favors this interpretation.

The participation of chromosomal proteins in G banding has been suggested^{11,12}. On the other hand, it has been reported that short exposures to NaOH result in G banding, longer ones giving only C bands⁹. Similarly, a 24 h incubation in $2 \times \text{SSC}$ at 65°C produces centromeric differentiation (see above), whereas a short treatment gives G banding¹⁰. G and C bands could then be related in a sequential manner by the successive extraction of protein components associated with DNA in variable degrees²⁰.

Résumé. Dans ce travail on présente des observations faites sur la coloration par le Giemsa de l'hétérochromatine centromérique de la souris. Les résultats permettent de supposer que des protéines chromosomiques entrent en jeu dans cette expérience.

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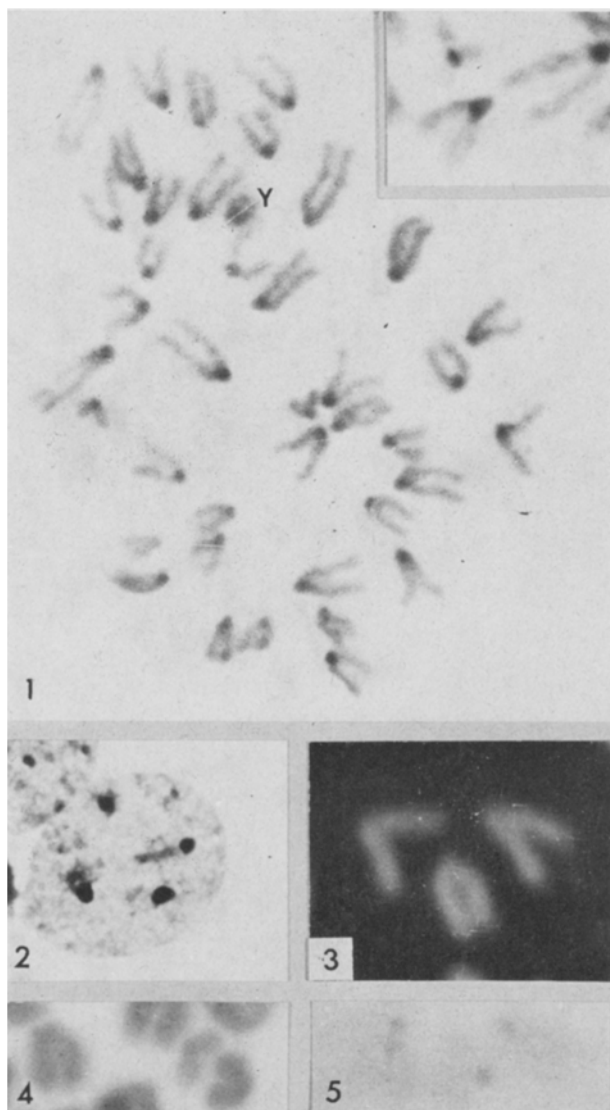


Fig. 1. Normal mouse metaphase after 2 min NaOH, Giemsa staining. Note the more intense staining of the centric regions. Y, the Y chromosome. Insert, similarly treated and stained chromosomes at a higher magnification.

Fig. 2. Nucleus, treated and stained as in Figure 1.

Figs. 3-5. Mouse chromosomes treated with NaOH. 3, stained with acridine orange. 4, stained by the Feulgen reaction. 5, treated with DNase and trichloroacetic acid, stained with Giemsa.

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²⁰ We wish to thank Dr. F. Rossi for his valuable discussion. This work was supported in part by grants from The Population Council, Inc., New York, and from the Consejo Nacional de Investigaciones Científicas y Técnicas (No. 4840/71), Argentina.

Effect of Specific Antibodies on *Neisseria catarrhalis* (lys⁻) Transformation Frequency

It has been reported by NAVA et al.¹ that immunization of rabbits with competent pneumococci stimulated production of antibodies which inhibited transformation. PAKULA^{2,3} showed similar results with the streptococci. He demonstrated that the antigenic structure of transformable streptococci, in the competent state, differs from the antigenic structure of cells in the noncompetent state. PAKULA³ reported that antibodies prepared against

noncompetent cells did not inhibit transformation of competent cells, and noncompetent cells treated with globulins prior to addition of competence provoking factor also inhibited transformation. These results therefore indicate that DNA-specific receptor sites on cell surface were blocked by antibodies to competent cells. Since competent cells contained an antigen specific for the competent state, and since this antigen appeared after the

competence-provoking factor has acted on noncompetent cells, Pakula hypothesized that the action of the competence-provoking factor may involve either unmasking of a hidden, preexisting antigen, or synthesis de novo of antigen. TOMASZ and BEISER⁴ found that prepared antisera against pneumococci, in their competent state, inhibited DNA-mediated genetic transformation as well as binding of radioactive DNA by the cells. The purpose of this investigation was to attempt to inhibit transformation of protrophy of a lys⁻ mutant of *Neisseria catarrhalis* by specific agglutinins against whole cells.

Materials and methods. Antibody against *N. catarrhalis* strain NE-11 was prepared by injecting a formalin-killed washed suspension intravenously into rabbits 4 times a week for a total of 4 weeks. Blood was collected from rabbits by way of the marginal ear vein 7–10 days after the final inoculation of the vaccine.

N. catarrhalis auxotrophic mutant was prepared for adsorption in the same manner as described for transformation by OTERO⁵ except that calf serum was not added to the transformational mixture. The pretrans-

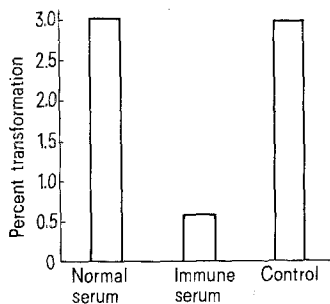
formational suspension of cells were exposed to 0.5 ml of undiluted immune serum for 1 h at 42°C prior to the addition of wild-type DNA. After the period of adsorption, transformational procedures and frequencies were performed in the same manner as described by OTERO⁵.

Results and discussion. The results show that a loss of transformants, approximately 80%, in the immune serum occurred (Figure). There appears to be little activity in the normal serum against *N. catarrhalis* NE-11 lys⁻. These results indicate that a specific substance present on the surface of the cell which was blocked (the cell viability of antibody exposed cells was the same as unexposed control cells) by specific agglutinins is essential in allowing the penetration of WT DNA.

Zusammenfassung. Antikörper mit einer gegen kompetente *N. catarrhalis* gerichteten Spezifität vermindern deren Transformationshäufigkeit bedeutend. Diese Beobachtung könnte auf die Existenz von Membranrezeptoren für transformierende Wildtyp-DNS hinweisen.

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Loss of transformants in the immune serum.

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Chromosome Analysis and Meiotic Behaviour of *Pappogeomys* (Cratogeomys) merriami merriami

The study of the geographic variability in fossorial rodents from the cytogenetic point of view, is of special interest because of the particular mode of life, which allows different types showing severe isolation and expressive variations of the chromosome complement both inter and intraspecifically, as described in several genera: *Spalax*¹⁻⁶, *Ctenomys*^{7,8}, *Thomomys*⁹⁻¹³, *Geomys*¹⁴ and *Pappogeomys*¹⁵. The aim of this paper is to report the first results of cytogenetic analysis of fossorial rodents restricted to the transverse volcanic system of México (Valley of México), initiated by the Merriami group of *Pappogeomys* (Cratogeomys): *Pappogeomys merriami merriami* located at the SE of the Valley.

Material and methods. A total of 8 specimens of *Pappogeomys* (Cratogeomys) *merriami merriami* (4 males and 4 females) collected at the National School of Agriculture, Chapingo, México State, were analyzed. The animals were injected with 1.0 ml/100 g body weight of a 0.04% colchicine solution and 2.30 h after they were sacrificed. Chromosome spreads from bone marrow, spleen and testes were obtained and permanent slides were prepared following the routine techniques^{16,17}. In each animal no fewer than 30 metaphases from each processed tissue were analyzed. The chromosome classification was made according to LEVAN et al.¹⁸ and AL-AISH¹⁹ criteria.

Results. The specimens of *Pappogeomys merriami merriami* studied showed a diploid chromosome number

$2n = 36$ and a fundamental number $NF = 66$. The complement shows 17 pairs of autosomes (16 pairs were biarmed and only 1 pair was acrocentric): 4 pairs of sub-

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