méthodologiques l'analyse expérimentale des mécanismes de la différenciation sexuelle des gonades chez le Triton *Pleurodeles waltlii* Michah.

Summary. Thanks to a method established by HUMPHREY[3] in *Ambystoma sp.* and founded on the orthotopic transplantation of the lateral mesoderm corresponding to the gonad primordium, we have produced, in the Salamander *Pleurodeles waltlii* Michah., 1 female which gives rise uniquely to female individuals. In this species, it is also possible to obtain, by an oestrogenic treatment during the larval stages of development, neo-females; these are genetic males feminized into phenotypic and perfectly functional females and their offsprings are uniquely composed of males[2]. The ability to experiment at will with unisexual, male or female, offsprings allows us to attack from a new basis, before any perceptible gonadic sex differentiation, the analysis of the mechanisms of this differentiation in the Salamander *Pleurodeles waltlii* Michah.

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Observations on the Staining of Centromeric Heterochromatin with Giemsa

Since the observation of PARDUE and GALL[1] of a denser staining of the centromeric regions with Giemsa as a result of the experimental sequence used to anneal satellite DNA to mouse chromosomes, techniques to reveal centromeric heterochromatin after denaturation-reassociation procedures have been developed[2,3]. It has been suggested that the Giemsa stain demonstrates reassociated (repetitive) versus non-reassociated DNA[2–6]. It is noteworthy that Giemsa-stained regions, in addition to the centromeric ones (G bands)[7,8–10], can also be revealed by other procedures where a denaturing-reassociating mechanism cannot possibly be involved[11–14]. Our observations on in situ DNA denaturation and reassociation, as revealed by acridine orange fluorescence[15], are also not consistent with the assumed interpretation. In the present experiments, the mechanism of the centric heterochromatin staining by Giemsa is investigated.

Material and methods. Standard flame-dried preparations of thymus and bone marrow cells of C3H/BA inbred mice and of lymphatic cells of mice bearing transplantable leukemias were employed. In some cases, a postfixation of the slides in 4% formaldehyde or 2.5% glutaraldehyde for 4–18 h was made. Slides were treated with 0.07 *N* NaOH, rinsed in running tap water, dried at 37 °C and stained either with Giemsa (1:10 in phosphate buffer at pH 6.8) for 10–15 min, acridine orange (AO) or by the Feulgen reaction. Some preparations were treated with DNase (2 mg/ml in phosphate buffer at pH 6.0) at 37 °C for 4–5 h, followed or not by 5% trichloroacetic acid (90 °C, 15–20 min) before staining.

Results and discussion. The treatment of mouse chromosomes with 0.07 *N* NaOH for 1–2 min, without further incubations in special conditions, results in a much denser staining of the centromeric regions with Giemsa, in relation to the arms (Figure 1). Very conspicuous interphase chromocenters are also observed (Figure 2). After a longer exposure (4–5 min), the chromosomes present a swollen and empty appearance although the contrast between centric regions and arms is enhanced; a variable proportion of metaphases is severely damaged.

Nuclei and chromosomes of preparations, treated 1–2 min with NaOH fluoresce green if stained with AO, indicating a double-stranded condition of DNA; only a few nuclei and metaphases show a yellow or red-orange emission. No differences in color or intensity of fluorescence between centromeric regions and arms are apparent (Figure 3). The Feulgen reaction also fails to reveal any differential staining of the centromeric regions (Figure 4). A 12–24 h incubation in 2 × SSC at 65 °C of slides treated with NaOH (1–2 min) enhances the intensity contrast between centric regions and arms in Giemsa-stained chromosomes. In these conditions, AO does not demonstrate any difference in color or intensity of fluorescence along the chromosomes in the majority of metaphases; this result is in disagreement with the findings of DE LA CHAPELLE et al.[16]. Remarkably, the incubation of untreated preparations also results in a heavier centromeric staining with Giemsa; in some metaphases, a banding pattern along the chromatids is also noticeable.

In aldehyde-postfixed preparations, the exposure to NaOH leads to no centric differentiation with Giemsa, the chromosomes staining uniformly, with an intensity equal to that of control slides (not postfixed nor subjected to NaOH). We have previously reported[15] a similar lack of centromeric differentiation with Giemsa in aldehyde-postfixed chromosomes subjected to heating (a procedure also used to reveal contromeric heterochromatin, 3).

On the other hand, the differential staining of the centric regions with Giemsa is also positive if the NaOH treatment is followed by DNase incubation (Figure 5). Prolonged staining times (up to 4–6 h) are however required. DNA extraction was controlled with AO in similarly treated preparations.

The present and previous[15] results indicate that the centromeric differentiation demonstrated by the Giemsa stain after exposure to heat or alkaline solutions is independent of the strandedness of chromosomal DNA as revealed by AO. That the treatment with NaOH in the time limits studied does not produce an unequal extraction of DNA from the arms in relation to the centromeric

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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.

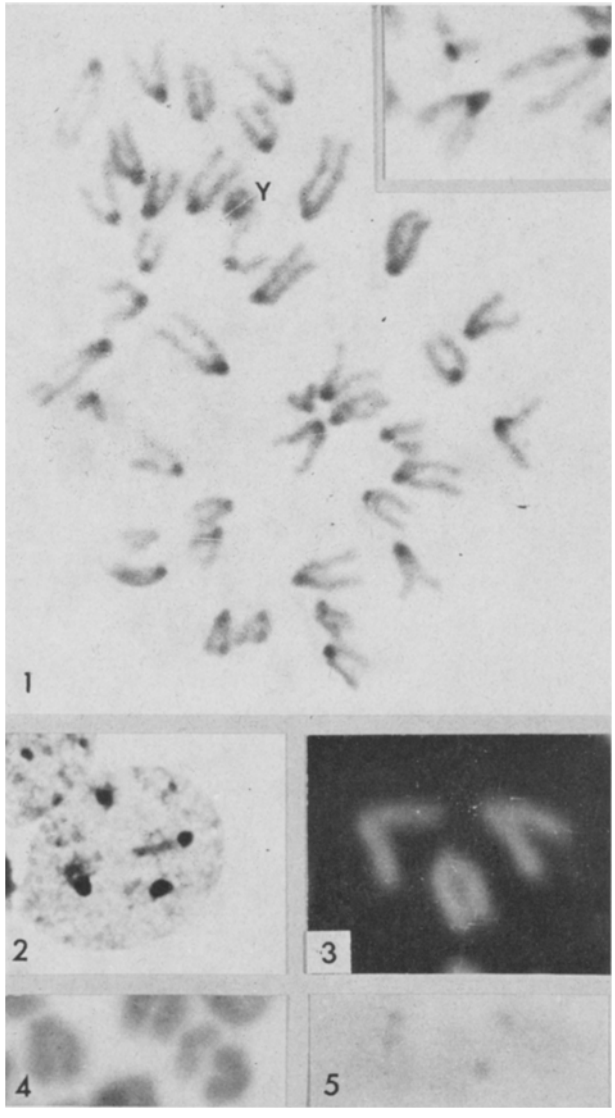

Fig. 1. Normal mouse metaphase after 2 min NaOH, Giemsa staining. Note the more intense staining of the centric regions. *Y*, the *Y* chromosome. Insert, similarly treated and stained chromosomes at a higher magnification.
Fig. 2. Nucleus, treated and stained as in Figure 1.
Figs. 3–5. Mouse chromosomes treated with NaOH. 3, stained with acridine orange. 4, stained by the Feulgen reaction. 5, treated with DNase and trichloroacetic acid, stained with Giemsa.

The high protein content of metaphase chromosomes[17] 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 suggested[18] that the latter is more firmly bound to chromosomal proteins. The fact that aldehyde postfixation, which binds chromosomal components in close association, allows DNA denaturation[15] 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 bands[9]. Similarly, a 24 h incubation in 2 × SSC al 65 °C produces centromeric differentiation (see above), whereas a short treatment gives G banding[10]. G and C bands could then be related in a sequential manner by the successive extraction of protein components associated with DNA in variable degrees[20].

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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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. PAKULA[3] 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