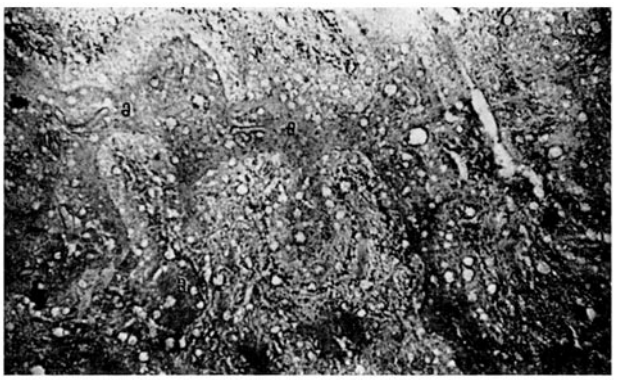

Fig. 2. The spleen of a rat ♀ aged 3 weeks, phosphate buffer, incubation for 90 min. Positive reaction in the lymphatic bands (a), the perifollicular parts react more faintly than in Figure 1 (× 33).

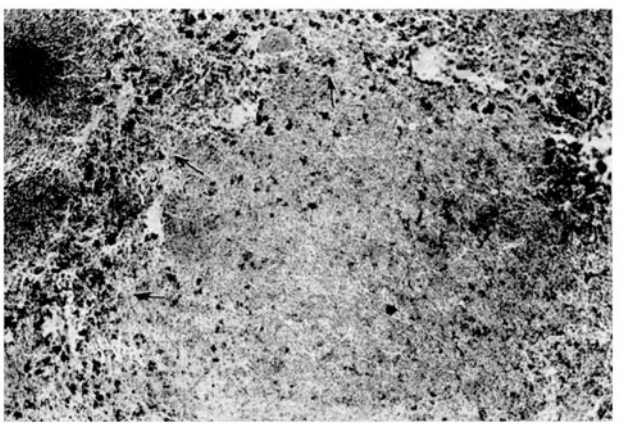

Fig. 3. The spleen of a mouse ♀ of 9 months, acetate buffer, incubation for 120 min. Positive reaction in the lymph node around which is seen an incomplete, strongly positive 'perifollicular halo' (arrows) (× 100).

established regularly in mouse spleen[3,6,10] and these islets produce a positive reaction in the area of red pulp.

Mouse spleen represents phylogenetically an older type of spleen dominated by lymphatic tissue[10]. In rat spleen the proportion of red pulp is greater and the lymphatic tissue is accumulated in bands around the central arteries. These lymphatic nodules showed a similar positive LAP reaction to that of mouse spleen. The 'perifollicular collar' is distinctly formed in rats and there are seen in it reticulum cells arranged in a circle and giving a positive reaction. Around these cells are other reticulum cells

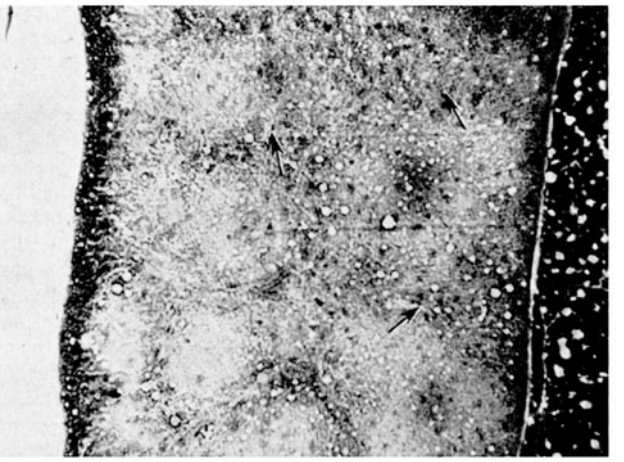

Fig. 4. The spleen of a mouse ♀ aged 2 weeks, acetate buffer, incubation for 120 min. Positive reaction only in the central arteries and close to them in the lymphatic tissue (arrows). The reaction is considerably fainter than in the pancreatic tissue seen on the right (× 33).

arranged in a loose circle forming a 'perifollicular halo' which reacts more weakly. Schweigger-Seidel's sheathed arteries were not encountered in the spleen of mouse and rat.

Comparison of the spleens of animals of different age showed that the positive LAP reaction was fainter in younger animals and, especially in mice, occurred in a narrower zone around the central arteries. Compared with the older animals, the difference was especially clear in the spleens of mice under 2–3 weeks of age and of rats less than 3 weeks old. This observation concurs with findings concerning the phases of development of the lymphatic tissue of the spleen[10].

Zusammenfassung. Leucinaminopeptidase wird histochemisch in der Milz von Mäusen und Ratten untersucht. Eine besonders ausgeprägte Reaktion zeigte sich in der Zentralarterienwandung und in den umgebenden Reticulumzellen der weissen Pulpa. Im Bereich der roten Pulpa verhalten sich nur die Inseln positiv, während eine extramedulläre Hämatopoese vorkommt.

L. K. Korhonen and S. Ruponen

Department of Anatomy, University of Turku (Finland), May 10, 1962.

[10] P. Cohrs, R. Jaffe, and H. Meessen, *Pathologie der Laboratoriumstiere* (Springer Verlag, Berlin 1958), I. Bd., p. 330.

Microchromosomes in the Embryonic Mitosis of the Domestic Fowl

Although recently there have been a number of publications on the somatic chromosomes of higher vertebrates, yet curiously enough only a few reports are available on the somatic chromosomes of the domestic fowl. Earlier workers like Lecaillon[1] and Hance[2,3], probably handicapped by the inadequate techniques and inherent refractory nature of the bird chromosomes, recorded a comparatively smaller and variable number of chromosomes than the later workers who counted as many as 66 (White[4]) and 78 (Ohno[5]) chromosomes in the somatic cells of the domestic fowl.

[1] A. Lecaillon, C. R. Soc. Biol. *69*, 31 (1910).
[2] R. T. Hance, J. Morph. Physiol. *43*, 119 (1926).
[3] R. T. Hance, Biol. Bull. *51*, 143 (1926).
[4] M. J. D. White, J. Genet. *26*, 345 (1932).
[5] S. Ohno, Chromosoma *11*, 484 (1961).

Japanese workers, as for instance YAMASHINA[6], have observed in the mitotic figures of the male eight pairs of large macrochromosomes surrounding 62 microchromosomes. NEWCOMER[7,8], working on the mitotic and the meiotic chromosomes of the fowl has, however, deemed it appropriate to relegate the microelements to the status of nonchromosomal bodies or 'chromosomoids'. Recent studies by VAN BRINK[9], KRISHAN[10] and OHNO[5] on the bird chromosomes in general and fowl chromosomes in particular have, on the other hand, established beyond doubt that the microchromosomes are in no way nonchromosomal, though they are very small in size and have a strong tendency to clump.

The present investigations have been made on embryos of 36–72 h incubation, pretreated in 0.25% colchicine for 10–20 min and subsequently squashed in aceto-carmine. Temporary as well as permanent preparations were studied and photographed under a phase contrast microscope.

Macrochromosomes: The present study has revealed six pairs of macrochromosomes (Figure 1) easily distinguishable from the microchromosomes. The macrochromosomes of the first two pairs, in order of size, are J-shaped bodies with a prominent submedian bend. In some premetaphase plates they appear as large rods with a slight constriction in the submedian region (Figure 4). Chromosomes of the third pair are rod-shaped elements with no visible constriction or bend. Macrochromosomes of the 4th pair are also J-shaped but with a diminutive small limb (Figure 6). Chromosomes of the fifth pair are the only V-shaped with a median bend in the complement and have been described as the sex chromosomes by SUZUKI[11]. In the female complement they are represented by a single V-shaped body (Figure 6). Chromosomes of the sixth pair are small rods and in many plates can hardly be differentiated from the larger of the microchromosomes.

Microchromosomes: YAMASHINA[6] has observed 62 microchromosomes besides the eight pairs of macrochromosomes, whereas NEWCOMER[7,8] recognizes only six pairs of macrochromosomes and a variable number of nonchromosomal chromosomoids. In the present study, due to the smaller size of somatic chromosomes as compared with those from the embryonic gonads, the 7th and 8th pair of macrochromosomes could not be effectively differentiated from the larger of the microchromosomes.

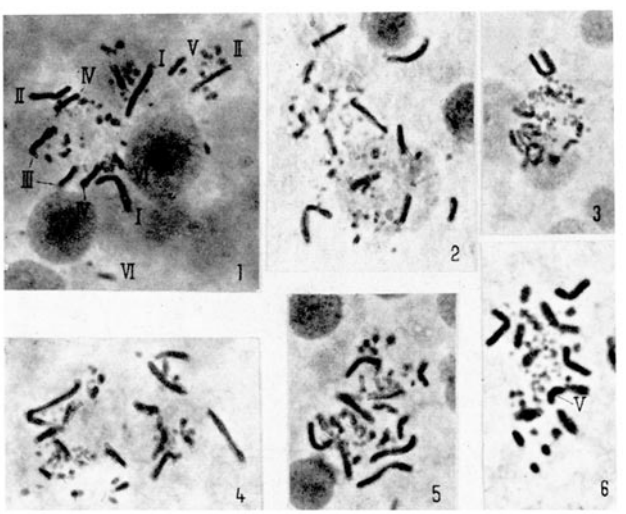

Fig. 1. Metaphase plate with 72 chromosomes. ×850. Fig. 2. Focussing on this preparation has revealed 72 chromosomes (12 macro and 60 microelements). Some of the larger microchromosomes show a double chromatid or dumb-bell structure. ×816. Fig. 3. Chromosome plate with 63 elements. ×725. Fig. 4. Early mitotic plate with 63 elements. ×850. Fig. 5. Premetaphase plate showing discrete macrochromosomes with a partial clumping of the microchromosomes. Focussing on this plate has revealed 69 elements. ×907. Fig. 6. Metaphase plate from a female embryo showing the single 5th V-shaped sex chromosome. ×1139.

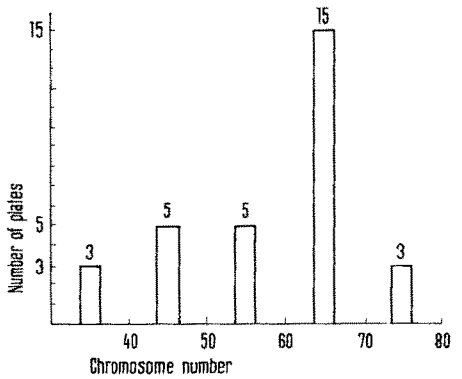

Fig. 7. Idiogram showing the range of numerical variation in the somatic chromosomes. Nearly 50% of the plates show numbers between 60 and 70.

Microchromosomes vary in size from the slightly elongated spherules to the minute dots. In some of the plates they fuse to form aggregate masses (Figures 5 and 6). This phenomenon is surely to be taken as an artifact and not as pointing to the nonchromosomal nature of these bodies. In Figure 2 some of the larger microchromosomes can be seen to show a double chromatid structure much like the larger macrochromosomes, while some other microchromosomes look like dumb-bells. This observation can be interpreted to mean that the microchromosomes regularly divide like the macrochromosomes.

Inconstancy of chromosome number: Counting analysis of 30 well separated plates has revealed a wide range of numerical variation in the somatic mitosis. Whereas the lowest recorded number is 39 (11 macro- and 28 microchromosomes), the highest is 76 which falls short of the male diploid number by two elements. No cell with subhaploid (less than 39) was ever encountered in the present study.

Numerical variation data, collected from plates showing no apparent trace of overlapping or clumping, has been presented in Figure 7. A majority of the plates (50%) reveal numbers in the range of 60 to 70. Incidently this range of variation is one of the highest reported so far from the embryonic tissues of the domestic fowl.

In a majority of the plates there has apparently been no variation in the number of the macrochromosomes, the six pairs (except in female where the 5th is unpaired) being regularly present. Even the plate with 39 elements reveals a complete set of macrochromosomes. Inconstancy in the chromosome number is, therefore, mainly due to the fluctuation in the number of the microchromosomes. Because of the technical difficulties it is not possible to ascertain with certainty, whether this inconstancy is inherent in the material, like the mammalian pathogenic

[6] M. Y. YAMASHINA, Cytologia *13*, 270 (1944).
[7] E. H. NEWCOMER, J. Hered. *48*, 227 (1957).
[8] E. H. NEWCOMER, Cytologia *24*, 403 (1959).
[9] J. M. VAN BRINK, Chromosoma *10*, 1 (1959).
[10] A. KRISHAN, M. Sc. (Zool. Hons. Sch.) Thesis, Panjab Univ. (1959).
[11] K. SUZUKI, Jap. J. Genet. *15*, 44 (1939).

and nonpathogenic somatic material, or is caused by the clumping of some of the smaller microchromosomes.

YAMASHINA[6] has reported a constant diploid number of 78 and 77 chromosomes from the somatic and the germ cells of the male and female respectively. NEWCOMER[7,8] on the other hand, describes the microchromosomes as nonchromosomal, the diploid number for the male and female being 12 and 11 respectively. Recent studies by VAN BRINK[9], OHNO[5] and KRISHAN[10] have, however, established the chromosomal nature of these bodies, though their number, according to them, is not as constant as reported by Japanese workers. The range of numerical variation, according to VAN BRINK[9] and OHNO[5], is from 67 to 82.

It seems that the haploid chromosome number in the male germ cells of the domestic fowl is 39 and deviations from this number may be due to the clumping of some of the microelements. In fact KRISHAN[10] has recorded 39 chromosomes in more than 50% of the first meiotic metaphase plates and OHNO[5] has found the same number in all the ten plates he counted. Somatic cells, in contrast to the germ cells, show a great variation in the chromosome number, which cannot be attributed to clumping alone. This view is in contrast to that of MATTHEY and VAN BRINK, as reported by OHNO[5], who believe that the microchromosomes are not inconstant in their number but that their exact determination is a difficult technical problem.

It looks probable that the microchromosomes show a variation in their number which within certain limits is inherent in the material but beyond that is caused by our technical failure to prevent the clumping of these refractory bodies[12].

Zusammenfassung. Beschreibung der somatischen Chromosomen des Haushuhns nach Colchicinbehandlung. Die Chromosomenzahl schwankt zwischen 39 und 76. 50% der Chromosomenplatten haben Zahlen zwischen 60 und 70. Die Makrochromosomen zeigen keine Abweichung in der Zahl. Die Zahlvariationen sind hauptsächlich durch die Mikrochromosomen bedingt.

A. KRISHAN

Department of Zoology, Panjab University, Chandigarh (Punjab, India), April 14, 1962.

[12] I am highly thankful to Dr. G. P. SHARMA, Panjab University, for his kind supervision, helpful criticism and laboratory facilities.

Role of Cell Division in Gastrulation of the Hemiramphid *Dermogenys pusillus* Van Hasselt

Morphogenetical movements in gastrulation of the Cyprinodonts *Fundulus heteroclitus* and *Xiphophorus helleri* occur in a normal fashion in cases in which cytokinesis is prevented by treatment with the antimitotic alkaloid colchicine[1,2]. To establish whether a more general significance should possibly be attached to this phenomenon, a corresponding investigation was performed with the halfbeak *Dermogenys pusillus* Van Hasselt (Hemiramphidae). For this purpose pregnant females in the first phase of gestation were injected intraperitoneally with 0.5 ml of 0.001 *M* colchicine solution. As in *Fundulus* and *Xiphophorus*[2] during gastrulation in stage 12 (beginning gastrula), 13 (advanced gastrula), 14 (further advanced gastrula) and 15 (closure of blastopore) both treated and control females were killed by decapitation and the ovaries were fixed in Bouin's fluid. The blastoderms were sectioned at 5 μ and stained with Heidenhain's iron haematoxylin. The mitotic indices representing the ratio of cells in mitosis to the total number of cells and expressed as %, were determined in each blastoderm on the basis of cell counts of the sections. As in *Fundulus*[1] and *Xiphophorus*[2], also in *Dermogenys*[3] the colchicine treatment did not have any apparent effect on gastrulation, which was similar to that in control blastoderms and consequently morphologically normal. In the colchicine-treated blastoderms, typical *c*-mitoses were observed. On the strength of the mitotic index determinations for colchicine-treated and control blastoderms, the number of *c*-mitoses increased, indicating that mitosis was completely blocked. The results are given in the Table. Thus, in stage 15 37.4% of the cells are in mitosis, while the mitotic indices of control blastoderms are relatively low during gastrulation. Consequently, also in early morphogenesis cell division can be dismissed as a causative factor and apparently provides only material for morphogenetic movements[4]. These results, corresponding very well with the findings of KESSEL[1] obtained with *Fundulus* and therefore of more general importance, are no doubt an argument for the view that gastrulation is a problem of mass movements and not of growth[5,6].

Mitotic indices of colchicine-treated and control embryos of *Dermogenys pusillus*. Stage 12 = beginning gastrula, 13 = advanced gastrula, 14 = further advanced gastrula, 15 = closure of blastopore.

Stage	Mitotic index (%) Colchicine treatment	Control
12	6.8	2.1
13	15.9	2.5
14	29.7	3.2
15	37.4	3.5

Zusammenfassung. Mit Colchicin wird die Bedeutung der Mitose für die Gastrulation des Hemiramphus-Fisches *Dermogenys pusillus* Van Hasselt aufgeklärt.

A. STOLK

Department of Histology, Free University, Amsterdam (The Netherlands), April 4, 1962.

[1] R. G. KESSEL, Exp. Cell. Res. *20*, 277 (1960).
[2] A. STOLK, Nature *192*, 371 (1961).
[3] O. J. EIGSTI and R. DUSTIN, *Colchicine* (Iowa State College Press, Ames 1955).
[4] J. HOLTFRETER, J. exp. Zool. *94*, 261 (1943).
[5] L. RHUMBLER, Wilhelm Roux' Arch. Entwicklungsmech. *14*, 401 (1902).
[6] W. VOGT, Wilhelm Roux' Arch. Entwicklungsmech. *120*, 384 (1929).