4911

NATIONAL LIBRARY
OTTAWA

CANADA

BIBLIOTHÈQUE NATIONALE
OTTAWA

NAME OF AUTHOR.....MOHAMMAD SHARIF AHMAD.

TITLE OF THESIS..CHROMOSOMES AND OOCYTE.....
...NUCLEI OF THE DOMESTIC...
...FOWL.....

UNIVERSITY.....of ALBERTA.....

DEGREE.....Ph.D.....YEAR GRANTED....FALL..1969..

Permission is hereby granted to THE NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(Signed).....

PERMANENT ADDRESS:

125...Sultanpur Bhawa
.ALLAHABAD, U.P. INDIA
.....

DATED...NOV..10....1969

NL-91

THE UNIVERSITY OF ALBERTA

CHROMOSOMES AND OOCYTE NUCLEI OF THE DOMESTIC FOWL

BY

© Mohammad Sharif Ahmad

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL, 1969

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Chromosomes and Oocyte Nuclei of the Domestic Fowl", submitted by Mohd. Sharif Ahmad in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

Robert T. Hardin
Supervisor

External Examiner

Date July 25, 1969

ABSTRACT

Mitotic and meiotic prophase (lampbrush) chromosomes, nuclear tonicity and nuclear morphology of Gallus domesticus were studied using light, phase, and electron microscopy; enzymes and autoradiography.

Although recent techniques were used, including the feather follicle procedure and podophyllin as an amitotic agent, an exact karyotype count of the mitotic chromosomes was not possible. However the results indicate the diploid count of metaphase chromosomes is in the upper seventies. Contrary to the Chromosomoid hypothesis, the microchromosomes behaved similarly to the macrochromosomes.

Meiotic prophase (lampbrush) chromosomes were studied in stained sections of growing and mature fowl ovaries and in oocyte (egg follicle) nuclei of laying birds. Procedures for the isolation of the fowl oocyte nucleus and its contents (lampbrush chromosomes and nucleoli) were developed.

Active oocytes, 18 days or older and larger than 150 μ diameter contain lampbrush chromosomes. Chromosomes in the remaining oocytes of growing birds or laying hens are in clumps, reticulum or diplotene configurations. Transformation of chromosomes from diplotene to lampbrush stage appears to be associated with activity of the Balbiani body in the cytoplasm. Lateral loops in the stained sections and in the isolated lampbrush chromosomes exhibited a linear array of points (chromatic nucleoli) presumably involved in nucleolar RNA synthesis.

Typical lampbrush chromosome bivalents were manually isolated from egg follicles, 6 to 3 mm diameter. In egg follicles above 3 mm diameter the nucleus is filled with only nucleolar lampbrush chromosomes and heteromorphic chromosomal fibers (elements) which exist until reduction division when clumped chromosomal masses are found. In this phase there is an unfolding of chromosomal strands (250 Å) synchronised with extensive synthesis of oocyte nucleoli. The results suggest that breakdown of lampbrush chromosomes is asynchronous.

Isolated nucleoli (solid spheroid, beaded ring, and pearl string) appear similar to those described for other animal oocytes. Giant nucleoli, 50 to 150 μ diameter, are present, inconsistently, in laying hen egg follicles of 0.6 to 2.5 mm diameter.

Isolated nuclei are similar to amphibian nuclei in their osmotic behaviour in KCl and sucrose solutions. The nuclear envelope, 350-450 Å thick, consists of two lamellae, each 100-125 Å thick, separated by a 175-200 Å interlamellar space and is covered by 600-800 Å nucleopores which collectively cover about 15% of the entire nucleus. The nucleopores are guarded by annuli on both sides of the envelope.

ACKNOWLEDGEMENTS

I wish to thank Dr. L. W. McElroy, Chairman of the Department of Animal Science, for the use of the Department facilities.

I am thankful to my supervisor, Dr. R. T. Hardin of the Department of Animal Science, for his help and encouragement and to Dr. R. F. Ruth of the Department of Zoology, who originally suggested the problem of the thesis, for his help and guidance during the study.

I am further indebted to Dr. R. T. Berg, Department of Animal Science; Dr. P. Bowen, Department of Paediatrics; Dr. W. Combs, Department of Animal Science; Dr. G. W. R. Walker, Department of Genetics; and Dr. J. Weijer, Department of Genetics, for their constructive criticism during the investigation and preparation of the thesis. I am grateful for the assistance and moral support of the fellow graduate students and staff of the Department of Animal Science.

Financial assistance from the National Research Council of Canada is gratefully acknowledged.

I particularly wish to thank my mother and my fiancee, Hamida Tufail, for their encouragement during the course of the investigation and preparation of the thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF COMMON ABBREVIATIONS

A.	Annulus
B.	Balbiani body
C.	Centriole
CG.	Chromatic (granule) nucleolus
CH.	Chromomere
CM.	Chromonema
CY.	Cytoplasm
EC.	Ectonuceoplasm
EF.	Egg follicle
EM.	Electron microscope
EN.	Endonucleoplasm
FM.	Follicular membrane
G.	Central granule
LL.	Lateral loop
M.	Mitochondria
N.	Nucleus
NL.	Nucleolus
W.	Nuclear (envelope) wall
Wh.	White of yolk
Y.	Yellow of yolk

INTRODUCTION

Much of the excitement in modern biology concerned with gene action and its physical and chemical nature, its arrangement, and operational behaviour, arises from genetic studies of prokaryotes whose chromosomes are simpler than of eukaryotes. Also there are many reasons why gene action and gene regulation in terms of present molecular models are insufficient to explain the same in higher organisms. Therefore, chromosome studies of higher organisms such as the domestic fowl (Gallus domesticus) may be informative.

Although the domestic fowl was used by pioneers in cytogenetics, e.g., Waldeyer (1870) who coined the term "Chromosome", and Holl (1890), the mitotic and meiotic chromosomes of the domestic fowl are still not well understood. The two most controversial problems concerning the mitotic (somatic) chromosomes: (a) diploid count of chromosomes and (b) number and function of microchromosomes, are still unsolved. Although a classical study on meiotic chromosomes of domestic fowl was made in 1904 by Hollander, very little is known about the meiotic chromosomes of the fowl.

Two types of chromosomes which have contributed to our understanding of gene action are: (a) giant chromosomes of the salivary gland of Diptera and (b) lampbrush chromosomes of vertebrates. Gall (1958) compiled morphological, physiological and genetic evidence suggesting that loops of amphibian lampbrush chromosomes corresponded with the gene loci. Also, Callan and Lloyd (1960) stated, "This makes it plausible to correlate site-specific morphologies of lampbrush chromosomes with the new adult phenotype". However our

knowledge of lampbrush chromosomes is derived exclusively from amphibia and reptiles on which only limited genetic information is available. On the contrary, much genetic information about the domestic fowl exists but little is known about its lampbrush chromosomes.

As early as 1904, the presence of lampbrush chromosomes in the domestic fowl was predicted from the stained section studies of oocytes by Hollander. However, no attempt has been made to isolate lampbrush chromosomes and other materials of the fowl oocyte nuclei as has been done in amphibians. Isolation of lampbrush chromosomes in the fowl may contribute towards relating genetic traits to specific chromosomes.

Therefore it was of interest: (1) to study the mitotic chromosomes, and (2) to isolate oocyte nuclei and their contents (lampbrush chromosomes and nucleoli), and obtain information about the oocyte nucleus necessary for such studies.

MITOTIC CHROMOSOMES

Literature Review

The earliest report of the chromosomes of the fowl was published by Holl in 1890. One of the major reasons for the many studies which have appeared since is that in the fowl, in addition to large chromosomes (macrochromosomes), there are many small elements (microchromosomes). Depending upon the interpretation of the investigator, these small elements may or may not be considered chromosomes. As the study of Newcomer and Brant (1954) represented a major development in studies of chromosomes of the fowl, published reports, summarized in Table 1, may be divided into two periods: (I) Pre-Newcomer (1906-1954), and (II) Post-Newcomer (1954-present).

I. Pre-Newcomer (1906-1954)

Reports during this period indicated the lack of agreement among investigators as to the diploid number of chromosomes. The reported diploid numbers ranged from a low of 12 (6 pairs) to a high of 80, (40 pairs). In his extensive studies on chromosomes in fowls of 17 breeds, Yamashina (1944) reported the number of macrochromosomes was 15 in the female and 16 in the male. In addition to these macrochromosomes, Yamashina claimed that both sexes had exactly 62 microchromosomes. However, there were very few workers who considered that the number of microchromosomes was constant and that an exact count could be obtained.

Other unsolved problems during this period were whether the female sex chromosomes were paired and which chromosome was the sex chromosome. Guyer (1909) reported the male to be heterogametic.

Table 1. Chronological tabulation of chromosome studies of the fowl

Authors	Year	Source and methods	Haploid number	Sex constitution	Sex chromosome and/or comments
		I. Pre-Newcomer 1906-1954			
Loyez	1906	Growing ovary, stained sections	6	-	-
Sonnenbrodt	1908	Growing ovary, stained sections	8-16 12*	-	-
Guyer	1909	Adult testes, stained sections	8-9	ZO	Largest, male heterogametic
Lecaillon	1910	Unreleased oocyte nuclei	12	-	-
Boring & Pearls	1914	Teste smears, stained sections	-	-	Refuted Guyer's work that male heterogametic
Guyer	1916	Embryo testes, stained sections	8-9	ZO	Large curved, in spermatocyte
Cutler	1918	Adult testes, stained sections	9-10	-	-

* Suggested number of pairs; ** Large chromosomes only

Table 1. Continued.

Authors	Year	Source and methods	Haploid number	Sex constitution	Sex chromosome and/or comments
Boring	1923	Adult testes, embryo gonads	9-11	-	-
Crew	1923	-	16	-	Largest
Shiwago	1924	Adult & embryo, gonad & soma	16	ZW	2nd largest, female heterogametic
Hance	1924	Gonad & soma culture smears	18-17(+1)	ZO	Largest, female heterogametic
Hance	1925	Embryo gonads & soma cultures	-	-	-
Hance	1926	Many organ muscles & gonad cultures	Prophase 30-35, Meta 18-17(+1)	ZO	Largest, J-shaped, single in female
Akkeringa	1927	Embryo gonads, stained sections	15-22	ZW	Largest
Goldsmith	1928	Embryonic gonads, stained sections	18	ZO	2nd largest, V-shaped, unpaired in female
Kemp	1930	-	18-19	-	-

Table 1. Continued

Authors	Year	Source and methods	Haploid number	Sex constitution	Sex chromosome and/or comments
Suzuki	1930	Embryonic gonads, stained sections	37-36(+1)	ZO	4th largest, V-shaped unpaired in female
Saguchi	1931	Embryonic gonads, stained sections	14(+1)-21	-	15 in Metaphase 21 in Prophase
White	1932	Adult testes, embryo ovaries	33(±1)	ZO	Largest, unpaired in female
Popoff	1933	Smears, embryo gonads, amnion	15-22 (+1) Meta, 30-35	ZW	Largest, female, heterogametic, number not consistent
Sokolow & Trofimow	1933	Embryo gonads & soma	16-35 25*	ZO	2nd largest, soma, 18-33, embryo gonads, 26(+1), unpaired in female
Unger	1936	Embryo gonads & soma	22-30(+1)	ZO	5th largest, unpaired in female
Sokolow, Tiniakow, & Trofimow	1936	Embryo gonads & soma, stained sections	as in 1933	ZO	5th largest, V-shaped, unpaired in female.
Oguma	1938	-	38	-	-

Table 1. Continued

Authors	Year	Source and methods	Haploid number	Sex constitution	Sex chromosome and/or comments
Miller	1938	Testes, ovatestes, stained sections	38-40	ZO	5th largest, unpaired in female
Scaccini	1944	Embryo gonads, stained sections	25-35	-	-
Yamashina	1944	Embryo gonads, stained sections	39-38(+1)	ZO	5th largest, unpaired in female, 17 breeds of domestic fowl
Newcomer	1952	Gonads, new smear technique	17±2	-	-
Brant	1952	A review. Concluded that variation in chromosome number was due to choice of material, method of fixation and staining.			
		II. Post Newcomer 1954 - Present			
Newcomer & Brant	1954	Growing till adult testes, developed a killing fluid	-	-	Renewed Sokolow's hypothesis, named the smaller chromosomes 'Chromosomoids'.
Makino et al.	1956	-	-	-	Favoured Yamashina's report

Table 1. Continued

Authors	Year	Source and methods	Haploid number	Sex constitution	Sex chromosome and/or comments
Brink and Ubbels	1956	Embryo gonads & spleens, squashes, pretreatment	39-38(+1)	ZO or ZW	Disputed Newcomer's hypothesis, sex constitution not sure.
Newcomer	1957	Gonads, pretreated culture & smear sections	6-5(+1)**	XO	Smaller number not consistent, insisted chromosomoid hypothesis correct
Brink	1959	Gonads, hypotonic pretreatment, squash	39-41 Maximum	ZO or ZW	Refuted Newcomer's hypothesis
Newcomer	1959	Testes, Feulgen stained smears	6-5(+1)**	-	Supported chromosoid hypothesis by meiotic chromosomes studies
Ohno	1961	Testes & soma, Feulgen stained squash	Upper 35, 39*	ZO or ZW	Disputed Newcomer's view
Ohno *et al.*	1962	Gonads, Feulgen stained prophase	-	-	12 or more microchromosomes associated with nucleolar organisor
Krishan, A.	1962	Gonads, squash	39 in male	-	Fluctuation of microchromosomes inherent in tissues

Table 1. Continued

Authors	Year	Source and methods	Haploid number	Sex constitution	Sex chromosome and/or comments
Donnelly, Donnelly & Newcomer	1962 1963	Chick bone-marrow plug, section, leucocyte culture, squash, thymidine autoradiograph	6-5(+1)**	ZO	Smaller are asynchronous to larger chromosomes, supported chromosomoid hypothesis.
Schmid	1962	Bone marrow culture thymidine autoradiograph	-	ZW	Larger one of microchromosomes, homologous to human Y in female
Poole	1963	Gonads, pin feather squash, phase microscope	14-15**	-	Macrochromosomes averaged 6-20, microchromosomes undiscernable
Stenius et al.	1963	Bone marrow gonads, squash	39-38(+1)	ZW	Confirmed Schmid, female ZW and not ZO
Ford & Woollam	1964	Testes, light & electron microscopy	38-40	-	Disputed Newcomer, syneptinemal complex in microchromosomes
Owen	1965	Spleen, thymus, bone marrow, hypotonic, air drying	39	ZW	Idiogram, paired chromatids of microchromosomes
Shoffner	1965	Review on the current knowledge of domestic fowl chromosomes.			

Table 1. Continued

Authors	Year	Source and methods	Haploid number	Sex constitution	Sex chromosome and/or comments
Shoffner & Krishan	1965	Chick feather pulp, hypotonic squash	39	ZW	Microchromosomes are genuine chromosomes, male ZZ, female ZW
Krishan & Shoffner	1966	Chick feather pulp, hypotonic squash	39	ZW	Established female constitution as ZW, and male as ZZ
Bammi *et al.*	1966	Ovary, hypotonic squash	-	ZW	Meiotic ZW do not pair during pachytene phase
Shoffner *et al.*	1967	-	39	ZW	Published detailed article
Bloom & Buss	1967	Different tissues (15) of embryo squashes, phase microscopy	40	-	Variation in the microchromosome number
Ray-Choudhuri *et al.*	1969	Colchicinized bone marrow cells	29(+1)-41(+1)	ZW	Identification of W chromosomes in the females of 5 species

This was immediately disproved by Boring and Pearl (1914), and later by Hance (1924, 1926), Goldsmith (1928), and Yamashina (1944), all of whom reported a ZO type of inheritance in the female. However, Shiwago (1924), Akkeringa (1927), and Popoff (1933) indicated the female had a ZW chromosomal arrangement. Guyer (1909, 1916) and Popoff (1933) claimed the sex chromosome was one of the largest chromosomes, while Sokolow et al.(1936), Unger (1936), Miller (1938) and Yamashina (1944) believed that the fifth largest chromosome was the sex chromosome.

II. Post-Newcomer (1954-Present)

Although there was little disagreement among researchers in the 10 years after Yamashina concerning the large chromosomes, considerable disagreement now exists regarding the nature and number of the smaller particles (microchromosomes).

In 1952 Brant indicated that variation in the reported chromosome number was not due to variation in chromosome number per se but mainly due to choice of material and methods of fixation, staining or preparing the tissue. Hoping to eliminate these sources of variation Newcomer and Brant (1954) used, on testicular tissue, air drying and smear technique in conjunction with a new fixative. They concluded that the smaller chromosomal particles were supernumerary rather than genuine chromosomes and therefore revitalized the idea of Sokolow and Trofimow (1933) that the smaller elements were inconsistent and probably auxillary chromosomes. Because the microchromosomes were variable in number and size, acentric, heterochromatic in staining, perhaps relatively inert genetically,

and exhibited coalescence or fragmentation at different stages of cell division, Newcomer (1957, 1959) termed these chromosomal particles "chromosomoids." Brink and Ubbels (1956) and Brink (1959), after attempting to count microchromosomes in several species of birds, concluded that the problem of microchromosome function and number could not be solved using only the light microscope.

Using bone marrow cells, leucocyte culture smears, squash preparations and autoradiography, Donnelly (1962) and Donnelly and Newcomer (1963), studied the mitotic chromosomes and found the microchromosomes synthesized DNA earlier than the macrochromosomes. This evidence supported Newcomer's view that the microchromosomes are not true chromosomes and their purpose may be to provide supporting substance for macrochromosomes.

However, results of other studies on fowl chromosomes do not agree with the idea that microchromosomes are not true chromosomes. Using the hypotonic technique and enzyme and squash preparations, Ohno (1961) studied meiosis and mitosis in germ and soma cells and concluded that total chromosomal number was in the upper 70's. Ohno based his conclusion on: (a) the presence of centromeres in the microchromosomes, (b) bivalency of the microchromosomes, and (c) the retention by the microchromosomes of their individuality during the complete process of meiosis and mitosis.

Krishan (1962a, 1962b) studied gonadal squash preparations from 36-72 hour male and female embryos and found the microchromosomes divided as regularly as the macrochromosomes. He suggested that the inconsistency in microchromosome number may be partially inherent in the material rather than due to the method of

preparation. Similar results were reported by Sharma *et al.*(1962).

Using light and electron microscopes, Ford and Woollam (1964) used the air dry technique to study meiosis and mitosis in testicular tissue. Their mitotic study showed the presence of centromeres and chromatids in the microchromosomes. In their meiotic study, in which they used the electron microscope, the microchromosomes showed pairing and syneptinemal complexes in primary spermatocytes during pachytene. Although the individual bivalents were clearly distinguishable, because of the variability of the microchromosomes they could not determine the exact number of chromosomes. However, Ford and Woollam supported Ohno's idea that during mitosis and meiosis microchromosomes do not differ from macrochromosomes in behaviour, but only in size.

Shoffner (1965) claimed that much of the variation in chromosomal number was due to some microchromosomes which are too small to be seen in the light microscope. In 1967 Shoffner *et al.* reviewed the methodology of avian chromosomal analysis and suggested methods of producing better mitotic plates and constructing idiograms, and established a system for describing the chromosomes of avian species based on size and centromere position.

Owen (1965), in his study of idiograms of different organs of chick embryos, concluded that the total karyotype was at least 78 in both male and female fowl. Bloom and Buss (1967) studied mitotic chromosomes from 15 different tissues of 2-16 day embryos and although they observed considerable variation in microchromosome number they concluded that total chromosome count in the domestic fowl is 80.

Another major problem has been whether the chromosomal composition of females was ZO or ZW (Brink and Ubbel 1956, and Brink 1959). However, Frederic (1961) and Schmid (1962) have demonstrated the presence of unpaired chromosomes among the larger elements of microchromosomes in females. Schmid (1962), using thymidine labelling, indicated one of the larger microchromosomes had a heavy and late thumidine uptake, was always single, and was consistently found only in the female. He considered this to be similar to the mammalian Y chromosome and therefore suggested that the female fowl was ZW.

Rothfels et al. (1963) described a W chromosome of the budgerigar of size order 9-10, suggesting that the sex determination mechanism in Aves may be ZZ, ZW. Also Ohno et al.(1964) found in females of six avian species a chromosome that was approximately 2/3 as large as the Z chromosome suggesting that the sex chromosomes in fowl may be ZZ, ZW. Similar results have been found by Owen (1965), Shoffner and Krishan (1956), Krishan and Shoffner (1966), Bammi et al. (1966), and Ray Choudhuri et al. (1969).

Another functional aspect of microchromosomes was shown from observing prophase preparations by Ohno et al. (1962). In these preparations twelve or more microchromosomes were associated with the somatic nucleolus. Shoffner (1965) also indicated that there were objects of non-random configuration in the preparations of karyological studies which were suggestive of some such organization.

Materials and Methods

White Rock chicks; 1, 2, 4, and 8 weeks old; from the University of Alberta Poultry Farm were used as the source of cells. Cells containing metaphase chromosomes were obtained using a modification of the feather follicle method proposed by Shoffner et al. (1967).

A mitotic arrestor, 0.05% solution of either Colcemid (CIBA) or podophyllin (Appendix I), was injected into the wing vein and/or body cavity at the rate of 0.08 ml per gram of body weight. Approximately 30-40 minutes later a primary wing feather was plucked. The follicle containing semisolid pulp was snipped 0.5 mm from the base and the pulp squeezed and transferred into a small clean test tube containing distilled water and kept at room temperature for 15-20 minutes for hypotonic treatment. The pulp was fixed by replacing the distilled water with a freshly prepared 45% acetic acid solution. After approximately thirty minutes the squash preparation began.

A brownish center or sometimes a distinctly transparent spot several microns in diameter was always observed among the translucent semisolid material of the pulp. As preliminary work indicated that more mitotic plates were obtained from this area than from other regions of the pulp a piece of pulp containing such a spot was removed from the fixative and touched to blotting paper to remove excess fixative. Using curved forceps the pulp was smeared with a circular motion on a clean slide to form a monolayer after which the larger pieces of cell debris were removed. Next a coverslip was placed over the material

and the slide placed on an even surface. As much thumb pressure as possible was exerted at one end of the coverslip. Working towards the other end of slide, pressure was applied to successive areas of the coverslip. A successful squash was indicated by the presence of interference color rings when the slide was held up to the light.

Using phase contrast, slides were examined to determine whether the desired configuration was present. Those slides which contained the desired configurations were made into permanent slides. To do this the temporary coverslip was removed by freezing the slide on dry ice. When the slide was frozen the coverslip was flipped off and the slide was stained using the Carbol fuchsin method of Carr and Walker (1961).

Slides containing squashed metaphase plates were subjected to enzyme action and/or tritiated Actinomycin D ($A.D.H^3$) autoradiography.

I. Enzyme Treatment of Squash Preparations

The squashed area of a slide was marked on two sides by two straight lines of fingernail polish. A coverslip was placed on the two lines (ridges) of fingernail polish, and on the edge of a piece of filter paper which formed the third side. The enzyme solution was fed by a long pipette on the fourth (front) side. Therefore a steady flow of enzyme solution across the area was maintained.

II. Autoradiography of Squash Preparations

The technique used for autoradiography of the squash preparations was basically that of Ebstein (1967). The squash preparation was brought into contact with a drop of $A.D.H.^3$ (sp. 2.5 c/mml; Schwarz Bio Res. Inc; Orange, N.Y.) in distilled water and exposed for

one hour. The squash was washed in a buffer solution and after dehydration with ethanol, the squash was coated with a NTB-2 emulsion.

Results

The mitotic chromosomes of the fowl during prophase, and metaphase are shown in Figs. 1, 2, 3, and 4. Observation of the plates indicates the large macrochromosomes and the small particles called microchromosomes.

Although the technique of studying chicken chromosomes by tissue sectioning had been perfected prior to the study by Yamashina (1944), investigators were not able to determine the exact chromosomal count. With development of newer procedures including squash preparation, hypotonic treatment, and blood and tissue cell culture, it was hoped that clarification of the chromosome number of the chickens could be made. Although the leucocyte culture technique has not been successful when used on the fowl, the development of the feather follicle method (Shoffner et al. 1967) provides a technique for obtaining cells for chromosomal studies.

Even though these newer procedures were used in this study, the number of microchromosomes varied between cells, and therefore it is not possible to specify an exact number of chromosomes (Table 2). However it appears that the total number of chromosomes, upper seventies and never more than eighty, agrees with the results of Ohno (1961), Ford and Woollam (1966), Bloom and Buss (1967), and Ray-Chou[illegible] et al. (1969).

Table 2. Diploid chromosome number of the domestic fowl

	Number of chromosomes					
	80	78	76	74	72	< 72
Number of cells	3	2	3	2	1	4

Shoffner et al. (1967) also used the newer procedures and found variation in chromosome morphology between homologues of the same cell, between chromosomes of different spreads of the same preparation and between different preparations. They suggested that the causes of such variations were differences in maturation, pre-treatment effects, distortions during squashing, and focal depth.

Because the number of microchromosomes was varying (Table 2), the question of whether these microchromosomes were chromosomes as claimed by Ohno and others, or not chromosomes as claimed by Newcomer and his group, required further investigation. Slides containing metaphase plates were exposed to trypsin, pronase, DNase, and auto-radiography. The results are shown in Figs. 5, 6, 7, and 8 for trypsin, pronase, and DNase.

Immediately after placing a few drops of trypsin or pronase on the slide the chromosomes appeared swollen and blurry (Fig. 5). Later the interchromatid spaces in both macro- and microchromosomes became clear. After either twenty-five minutes of trypsin action or four minutes of pronase action the core of the chromosomes and interphase cell nuclei were no longer visible and only the boundaries of chromosomes and interphase cells were visible (Fig. 6). Auto-

radiography of squash preparations showed that the boundary of the interphase cells and the mitotic chromosomes were covered with thick grains so that metaphase plates became difficult to detect (Figs. 9 and 10). Some slides which had been treated with proteolytic enzymes and others which had not been treated, were treated with DNase and exposed to autoradiographic treatment. The effect of DNase on the chromosomes which had been previously exposed to trypsin or pronase was so drastic that even the boundaries of the cells and chromosomes disappeared. After these slides were exposed to autoradiographic treatment, examination indicated that the chromosomes did not show any deposits of grains and also the interphase cells were free from grains.

Some slides were stained with a temporary acetic orcein stain. Later they were subjected to autoradiographic treatments. They also failed to develop grains.

The results of using autoradiography of chromosomes after using DNase or orcein, agreed with Ebstein (1967) that tritiated Actinomycin D can be used as a cytochemical label for small amounts of DNA.

Discussion

There was nothing in the behaviour of the microchromosomes which could be assigned as a point of difference between macro- and microchromosomes. The results of this experiment therefore agree with Shoffner (1965) and Krishan (1962a) that the inconsistency in the chromosomal count is mainly due to fluctuation in the number of microchromosomes. Whether this inconsistency is similar to that observed in the pathogenic and non-pathogenic soma cells of mammals and is inherent in the materials due to clumping of smaller

microchromosomes or a splitting of the microchromosomal chromatids during either squashing or using the mitotic arrestor is not known (Ohno, 1965). Krishan (1962a) has reported that the inconsistency was more obvious in somatic cells than gametic cells and that it was not due to clumping only.

Although Newcomer and Brant (1956), Newcomer (1957), and Donnelly (1962) have questioned whether the microchromosomes may be considered as chromosomes, there is now considerable evidence from studies on both meiotic and mitotic cells that there are no essential differences in structure and behaviour between the macrochromosomes and the microchromosomes (Ohno 1961, 1965; Krishan 1962a, 1962b; Ford and Woollam 1964; Owen 1965; and Shoffner 1965).

The microchromosomes retain their individuality during the process of meiosis and mitosis (Ohno 1961 and Sharma et al. 1962). By observing the synaptinemal complex which is a characteristic of pachytene chromosomes, Ford and Woollam (1964) observed bivalency of microchromosomes. In the present study it was observed that the microchromosomes divide as regularly as the macrochromosomes and have identical responses to enzyme actions. In some microchromosomes, double chromatid structures similar to those present in the macrochromosomes, and in other microchromosomes, dumb-bell type configurations, are supporting evidence that the microchromosomes divide as regularly as the macrochromosomes. The presence of kinetochores in some of the microchromosomes is additional evidence that microchromosomes behave as regular chromosomes. Whether this is true for the smallest microchromosomes remains to be determined. Electron microscope studies by Ford and Woollam (1964) indicated that some

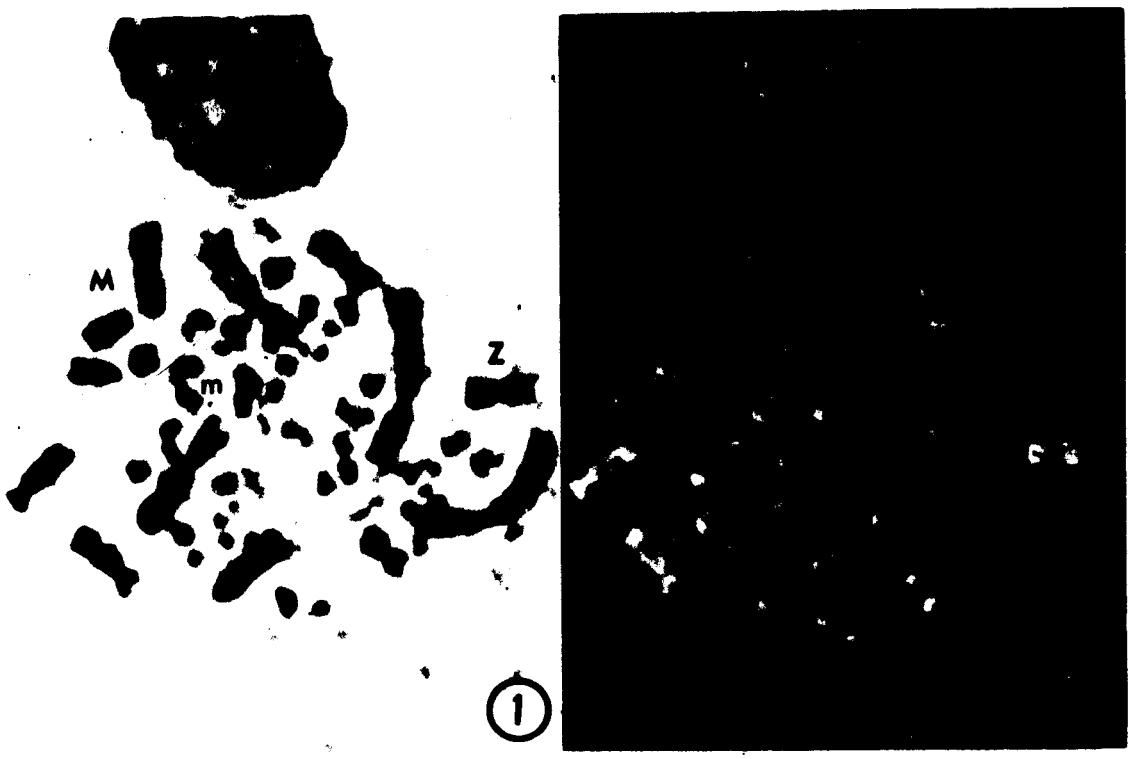
of the smallest microchromosomes contained less fibrillar material per unit of length than the large chromosomes. Therefore the very small microchromosomes may be less genetically significant.

If the microchromosomes were larger than the limit of resolution, then as in macrochromosomes, the length and kinetochore position could be used for identification (Shoffner et al. 1967). However, under present conditions they are not. Therefore, improvements in technique are still needed. One technique may involve isolation of early lampbrush chromosomes. For instance, it would be informative if oocyte nuclei could be isolated in such a stage that all the bivalents could be discerned in a typical diplotenic configuration. Low magnification transmission electron microscopy, or scanning electron microscopy may be of some advantage in identifying and counting the microchromosomes if well spread mitotic cells are obtained on monolayer spreads.

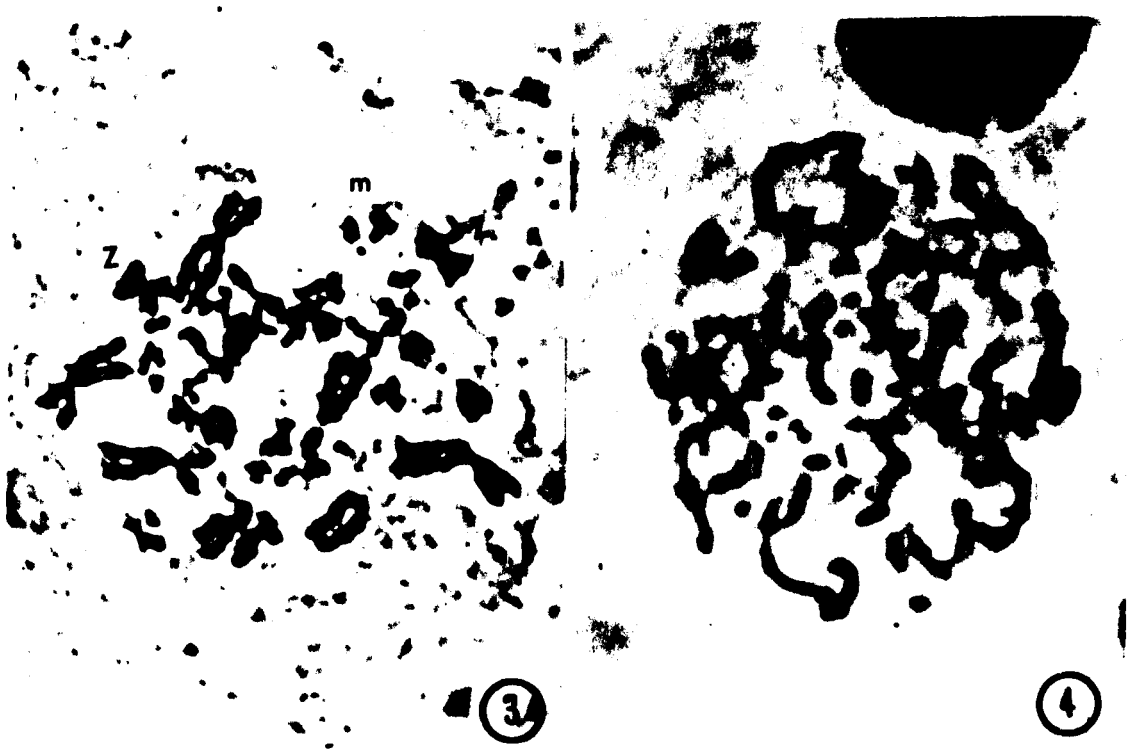
Figs. 1, 2. Metaphase plates from female feather follicle cells: Fig. 1. Plane optics showing macrochromosomes (M), microchromosomes (m) and a sex chromosome (Z). Podophyllin used as mitotic arrestor. Fig. 2. Phase optics, magnification approximately 3,000 X.

Fig. 3. Metaphase plate from male chick feather follicle cell, showing microchromosomes (M) and paired sex chromosome (Z), Colcemid used as mitotic arrestor. Magnification approximately 2,800 X.

Fig. 4. Prophase plate from feather follicle cell of growing chick. Podophyllin used as mitotic arrestor. Magnification approximately 3,000 X.



①



③

④

Figs. 5, 6. Metaphase chromosomes of feather follicle cell: Fig. 5. Immediately after trypsin placed on slide. Fig. 6. Same cell as in Fig. 5 after 25 minutes of trypsin action. Arrow indicates microchromosome which was affected similar to macrochromosomes. Magnification approximately 1,800 X.

Figs. 7, 8. Squash preparation of metaphase chromosomes: Fig. 7. Prior to treatment. Fig. 8. Same plate exposed to 4 minutes pronase and 2 minutes of DNase action. Arrow indicates area of mitotic plate dissociated by enzyme action. Magnification approximately 1,700 X.

Figs. 9, 10. Squash preparation of feather follicle cells: Fig. 9. Interphase cells showing a heavy labelling of tritiated Actinomycin D. Magnification approximately 600 X. Fig. 10. Autoradiograph plate showing dispersed tritiated Actinomycin D granules. Magnification approximately 1,750 X.

5

6

7

8

10

MEIOTIC (LAMPBRUSH) CHROMOSOMES

Literature Review

Although the domestic fowl was the species used by the pioneers of chromosome studies, Waldeyer (1870) and Holl (1890), the meiotic chromosomes of the fowl are still not well understood. Meiotic chromosome studies of the domestic fowl were a major area of cytogenetic research in the late nineteenth and early twentieth century (Waldeyer 1870, Holl 1890, Hollander 1904, Loyez 1906, Sonnenbrodt 1908, Durme 1914, Boring and Pearl 1914, Hance 1926, Goldsmith 1928, Miller 1938 and Sluiter 1939). After 1939 there was very little research on the meiotic chromosomes of the fowl until 1963 when Hughes, utilizing information reported by Ohno (1961), reconfirmed the original thesis of Waldeyer (1870).

The onset of meiotic prophase begins at 14-15 days of incubation (Hollander 1904, Loyez 1906, Sluiter 1939, Durme 1914, Romanoff 1960, and Hughes 1963). Although it is now well established that oogenesis in the domestic fowl has reached the diplotenic phase at the time the chick is hatched (Sluiter 1939 and Hughes 1963), a major question remains as to what happens to the chromosomes during further maturation (post hatch ovulation) of the oocytes. Hollander (1904) considered the chromosomes to be in a resting stage while Durme (1914) referred to them as being in a temporary pause prior to the next growth stage. A controversy existed between Loyez (1906), Sonnenbrodt (1908), Durme (1914), and Goldsmith (1928) concerning the resting stages of oocytes and presence or absence of chromosomal structures during advanced growth stages of oocytes.

The four stages of nuclear transformation in oocytes obtained from fowl 4 days old to 20 days old proposed by Hollander (1904) are: (i) The nucleus in the pseudo-reticulum was slightly modified from that of four day chicks and its volume increased. The nucleus contained chromatic and voluminous nucleoli in an eccentric position. The branched filamentous structure formed the chromatic apparatus; (ii) The nucleic filaments thickened and formed a network. This process started in the center of the nucleus and continued towards the periphery; (iii) The chromatic segments became uniformly coarser and thicker and the nucleoplasm filled with nucleic grains. The nucleus at this stage was surrounded by a double membrane in which the inner layer was a proper nuclear membrane, and the outer layer a cytoplasmic membrane; (iv) The barb nucleus was formed by chromosomes with lateral projections. Although genesis of the barb nucleus was not clear, it was associated with a very long diplotenic phase.

Loyez (1906) described six stages of nuclear transformation. Some of his conclusions were: (i) Chromosomes appeared similar to a ball of long chain folded over interwovenly. Later chromosome threads fragmented and formed a series of rings and rectangles; (ii) Stainable barb (lampbrush) chromosomes in the granulated nucleoplasm were covered with double membranes - the nuclear membrane and inner cytoplasmic membrane; (iii) Barb (lampbrush) chromosomes became less visible and less stainable as a result of fragmentation and disintegration into grains. These grains later reunited in an advanced growth stage of the oocytes and formed new chromosomes. No nucleoli were observed at this stage; (iv) The new chromosomes formed loops and coils and migrated to the center of the nucleus; (v) Each chromosome condensed and

together the chromosomes formed an irregular chromatic mass in the center of the nucleus. Later small nucleoli appeared near this chromatic mass, and finally; (vi) The chromatic mass formed loose coils of filaments and rings. The nucleoli disappeared and the grains were expelled out of the nucleus.

Sonnenbrodt (1908) repeated Hollander's and Loyez's work. She described twelve stages during this growth period and her results agree with Hollander. On the basis of her observations, the last seven stages during intrafollicular growth of oocytes may be summarized as: (vi) The nucleus was in the pseudo-reticulum stage as described by Hollander (1904) and was filled with chromatic grains. The nuclear reticulation gradually increased; (vii) Chromosomes transformed to either a streptococcal type ring or a barb like appearance; (viii) The nucleolus, which was already present, disintegrated; (ix) Chromosomes completely disappeared. The granulations of barb chromosomes aligned as parallel rays; (x) The chromatic nucleoli were formed at the expense of chromosomal granules (chromatic grains) and increased in volume and number; (xi) The chromosomes were regenerated at the expense of chromatic nucleoli and (xii) Before maturation, chromosomes formed coils and loops.

A classical study of oogenesis of mature birds was made by Durme (1914). Although her special interest was to extend the work of Hollander (1904) on the transformation of nuclei and chromosomes, she concentrated on the sparrow and swallow rather than the chick embryos and the very young chicks that Hollander (1904) had used. She concluded that the growth and maturation of the intra-follicular oocyte was associated with nuclear transformations and was divided into three

phases of vitellogenesis.

Durme agreed with the description of Hollander regarding the meiotic prophase structure of chromosomes which existed in the chicks until four days old and which may be considered as the condition existing prior to the first stage of vitellogenesis. She concluded that in the first of the three phases the nucleus had a large nucleolus and the chromosomes were transformed to chromatic rings or undivided filaments having branches throughout the nucleus. At a later stage of pseudo-reticulum, the central nucleoplasm was covered with the chromatic segments which were transversely striated. The peripheral zone of the nucleoplasm became relatively clear. The pseudo-reticulum of the nucleus underwent an additional slow transformation leading first to a transverse division of chromosomes into segments, and thereafter the appearance of chromatic granules; secondly, the production of chromatic nucleoli; and lastly, barb (lampbrush) formation of chromosomes.

In the second phase of vitellogenesis she observed a double membrane surrounding the nucleoplasm, and an increased size and number of nucleoli. These nucleoli were smooth or attached to chromatic fragments. The chromatic segments were either still in a barb condition or were split longitudinally close to the nucleoli. Later, the entire nucleoplasm was covered with chromatic trabeculae and nucleoli of irregular shape and size.

In the third stage of vitellogenesis, the nucei were located close to the vitelline membrane. The chromosomal segments were reduced to globules, sometimes x-shaped. At the end of the growth phase, the nucleolus consisted of a large object of irregular shape and

size. This clumpy mass of chromatin, which she described as a mass of nucleoli, was the basic material for the first maturation division.

Unlike the sparrow and other birds, in the chicken the nucleoli of the oocyte nucleus were not spherical but masses of irregular shapes and sizes. Barb segments were adhering to the chromatic debris around the nucleoli. Durme observed irregular chromatic segments at all stages of growth of oocytes which never disintegrated to the extent that Loyez (1906) and Sonnenbrodt (1908) reported. Also she observed a mass of numerous fine chromosomal filaments in 10 mm diameter egg cells. An increase in number and size of multiple nucleoli was attributed to the chromatic filaments of the nucleus.

Goldsmith (1928) disputed the findings of Hollander (1904) and Sonnenbrodt (1908) and claimed that the nuclear changes occurring in the oocytes of 14-18 day chick embryos represented synizesis leading to leptotene phase. He also claimed that a long 'resting' phase occurred between hatching and 65-69 days of age, when the germ cells passed into pachytene phase and that during the final rapid phase of growth immediately before ovulation, a second 'resting' period follows without an intervening diplotene phase.

Related to these early studies are the findings of Koltzoff (1938), who published his results of forty years of work on the structural transformation of chromosomes and their participation in cell metabolism in the triton, pigeon and hen. He divided the process of transformation of chromosomes during oogenesis, including lampbrush form, into seven different stages. Although his physiological assumptions and hypotheses are antagonistic to the present concepts of biology, his cytological record is still a valuable piece of work which

has been neglected by biologists.

In the first stage of oogenesis in the fowl Koltzoff (1938) observed that initially the nucleus was one eighth of the volume of the oocyte. Diplotenic chromosomes could be detected in oocytes with diameters of 80 - 150 μ which contained nuclei from 26 - 40 μ. The chromosomes he sketched were in typical diplotenic configurations with crosses, circles and loops, demonstrating chiasma formations. A maximum of four chiasmata were resolved in the largest chromosomes. Situated along the axes of the bivalents were chromomeres of round and angular shapes and of variable sizes. He considered that chromosomes in this stage of development contained what he called 'primary chromomeres' which stayed with the chromosomes till the beginning of the third stage. The chromosomes had, however, lost the Feulgen reaction in the second period. In the third period chromomeres started spreading by either splitting or diffused lateral growth and transformed into nucleolar granules. At a later stage, 215 - 400 μ oocytes which had nuclei of 65 - 90 μ, the bivalents consisted of a long chain of diffused chromomeres, which extended horizontally and contained fine nucleolar granules. The chromosomal axis was described as segmented. It appeared similar to the vertebral column of fish.

In larger oocytes of up to 600 μ, with a nucleus 120 μ, a long axis of the bivalent was retained by the primary chromomeres in a discrete but straight line arrangement. The horizonatal lateral processes of the chromomeres (loops) extended in length and width and developed more granules of a larger size which stained weaker than the primary chromomeres. This process of granulation continued, resulting in larger nucleolar granules in the side loops. At this

stage the granules on the lateral loops of the lampbrush chromosomes were termed as secondary chromomeres.

During the fourth and fifth stages (oocytes 1400 μ, nucleus 310 μ) the side branches developed heavy granulation and simultaneously detached from the axes. The granules were scattered and floated free in the nucleoplasm, therefore leaving the primary chromomeres attached to the axis of the original diplotenic chromosomes. The remaining primary chromomeres were those which Koltzoff had termed as tertiary chromomeres. The chiasmatic loops of the original bivalents now were not symmetrical and they lost their nucleic acid content.

Later stages were concerned with diakinesis and first reduction division. The hypothesis which Koltzoff suggested is obvious from one paragraph of his conclusion.

> "The author holds that there is a marked difference between the genotype of the chromosome and its phenotype, which undergoes some changes in the same chromosome during various stages of the development. It is only the genonema that belongs to the genotype while chromoplasm, chromatin, etc., are phenotypic structures, and their changes do not influence the hereditary qualities of the genonema."

Sluiter (1939) studied stained sections of 15-day chick embryos and demonstrated three phases of nuclear changes which covered leptotene, zygotene, pachytene, and diplotene. In his third phase the nucleus stayed in the diakinetic stage with a fine chromatic reticulum and bilobed single or double nucleolus. This was the stage in which Sluiter (1939) believed the oocytes rested for a long period. However, he did not continue the study of chromosomal transformation during growth to the adult stage of the fowl to determine whether this was true. Olsen (1942) studied maturation and fertilization in hens and found that 24 hours before ovulation the wall of the germinal disc disintegrated.

Shortly before ovulation the chromatin, found in thin threads and scattered throughout the nucleus, became grouped in a smaller area near the centre of the nucleus. Then the first maturation division occurred with the first polar body formation.

The most interesting of meiotic chromosomes are the giant chromosomes with lateral projections which exist in the primary oocyte nuclei of most vertebrates, in some invertebrates, germ cells (Callan 1957), and probably in somatic cells (Nebel and Coulon 1962). These chromosomes were discovered by Flemming (1882). Because of the branch-like structures arising from their axes, Ruckert (1892) studying fish termed them as "lampbrush chromosomes", Hollander (1904) studying the domestic fowl described them as "barb chromosomes", and Duryee (1941, 1950) studying amphibians coined the term "lateral loop chromosomes". In this study these chromosomes will be referred to as lampbrush chromosomes. These structures first appear during the pachytene or diplotene stages of meiotic prophase and by throwing the main axis into lateral loops become highly developed as the oocyte enters the growth phase (Callan 1963).

There have been reports indicating the presence of lampbrush chromosomes in the oocyte nucleus of fowl from eighteen days embryo to maturity. Using stained sections of ovaries, Hollander (1904), Sonnenbrodt (1908), and Durme (1914), reported the presence of barb (lampbrush) chromosomes in the fowl. Koltzoff (1938), also using stained sections, studied the lampbrush chromosomes of chickens, tritons, and pigeons. Nebel and Hackett (1962), using electron microscopy, proposed the presence of lampbrush chromosomes in pigeon testes. Hughes (1963) in an ovarian squash preparation of a fourteen

day embryo observed a diplotene chromosome which she considered to be a lampbrush chromosome. However, no investigator has attempted isolation of the lampbrush chromosomes in the fowl. The experiments of Duryee (1937, 1941, 1950) in which he attempted to study chromosomes in situ in the isolated oocyte nucleus of amphibians resulted in a break-through in lampbrush chromosome study. Since then a large number of reports concerned with morphological studies of lampbrush chromosomes of amphibians and fish oocytes have appeared in the literature (Brachet 1940, 1942; Ris 1945, 1955, 1956; Lafontaine and Ris 1958; Dodson 1948; Callan 1952, 1957, 1963, 1966; Tomlin and Callan 1951; Guyenot and Danon 1953; Gall 1954a, 1956, 1966a; Callan and Lloyd 1960; Wischnitzer 1957, 1961; Sirivastava and Bhatnagar 1962; Miller 1964a, 1965a; and MacGregor 1965).

Recently Koecke and Muller (1965) succeeded in obtaining a squash preparation of the nucleus from fowl and duck oocytes and from phase observations reported the presence of lampbrush chromosomes in 2.5 - 4.5 mm diameter egg follicles.

Techniques of Isolation of Lampbrush Chromosomes

The basic technique for the manual isolation of lampbrush chromosomes in amphibian and urodele oocytes has been described by Duryee (1937, 1950), Gall (1954a, 1966a) and Callan and Lloyd (1960). However, bird oocytes are not as easily accessible as in amphibians and fishes because they are encapsulated in a number of thick follicular layers. Also in bird oocytes the relative content of the cytoplasmic area and its inclusions as compared with its nucleus is very high. Therefore the technique of isolating the nucleus in the fowl requires modifications in removing the thick layers of follicular membranes which surround the oocyte and in isolating the nucleus from yolky cytoplasm.

Isolation of the nucleus from the egg follicle is easier if the anatomy of the ovary and the histology of the egg follicle and the arrangement of its histological structures is understood.

In birds normally only the left ovary is persistent and functional. It is situated in the dorsal part of the abdominal cavity, and is attached to the dorsal wall by mesovarium and is opposite the last two ribs in the last intercostal space. It is in contact dorsally and anteriorly with the posterior end of left lung, dorsally with the anterior lobe of kidney, ventrally with the glandular stomach and spleen and medially with the posterior venacava. As shown in Figs. 11, 12, 13, 14, and 15 in younger birds the ovary appears as a leaf-like structure, with irregular and racimose borders and in adults full of grapelike pedunculate egg follicles.

The oocyte is limited by the zona radiata which lies next to

the vitelline membrane. These two layers appear late in the developing egg follicle and are not visible during isolation. Immediately surrounding the vitelline membrane is the layer of follicular epithelium (membrana granulosa) which consists of cuboidal cells in both smaller and larger egg follicles but in oocytes of intermediate size has a stratified and pseudo stratified appearance (Bellair 1965). The results of this study indicate that pseudo stratified epithelium of follicular layers appears in egg follicles ranging from 4.0-6.0 mm.

Distal to the follicular layer is the fibrous layer of extra cellular matter, the membrana propria, which is about one U deep and is normally not visible during isolation. Next are the closely packed cells of theca interna and the loosely packed cells of theca externa which are pervaded by blood vessels, collagen fibers, and luteal cells. The outermost covering is the peritonial wall which surrounds the follicle.

For convenience the technique of isolation is described in three stages: (I). Preparation of the tissue, (II). Isolation of the nucleus and (III). Isolation of lampbrush chromosomes.

I. Preparation of the Tissue

A. Removal of the Ovary.

Although it is possible to remove either a part of the ovary or the required size of the egg follicle without killing the bird, the birds were sacrificed because of the labour involved in handling the surgical procedures. After a bird was sacrificed a mid ventral incision was made. Another cut, left of the sternum (Mediastinum thoracis) passing through the last intercostal space towards the

vertebral column, was needed to expose the ovary. After snipping the mesentry and pushing the excised intestine aside with curved forceps the ovary was held at the base and the complete ovary was excised at the root without damaging the blood vessels.

The material obtained was immediately transferred to two or three sterile specimen bottles which were sealed with paraffin wax and stored in a refrigerator for later use. Shown in Figures 11 through 16 are isolated egg follicles (Figs. 11 and 12), a growing ovary (Fig. 13), portions of ovaries (Figs. 14 and 15) and isolated follicles (Fig. 16).

B. Location of the Nucleus.

After procuring the egg follicles, it was necessary to determine the position of the nucleus. In egg follicles up to 2 mm diameter (hereafter diameter = dia) the nucleus was either free to move or was loosely embedded in the follicular epithelium. The oocyte was normally translucent with the nucleus a transparent ball. Although in a few egg follicles of about 3 mm dia the nucleus was observed in the center of the oocyte, normally in large egg follicles, over 2 mm in dia, the nucleus was found embedded in a evaginated groove formed by the follicular epithelium (Fig. 12). The layers in immediate association with the oocyte, the corona radiata and vitelline membrane, surround the nucleus. Although the ooplasm began growing turbid in consistency and yellow in color as the egg follicles grew beyond 2 mm dia, at all times the nucleus was relatively transparent (Fig. 12). Localization of the nucleus was further assisted by cleaning the egg follicles in isolation media (Calcium free 0.1 M K/NaCl in 5:1 ratio;

Medium 'D' of Callan and Lloyd 1960).

C. Cleaning of the Egg Follicles

The egg follicle of the required size was removed from the dissected ovarian tissue and cleaned into a watchglass containing (5:1) isolation medium. The outer membrane of the egg follicle (tunica vaginalis) or the peritonium, including the network of the blood vessels underneath, was removed with a pair of forceps. In smaller and larger egg follicles as compared to medium size egg follicles (4.0 - 6.0 mm), the nucleus was observable only after a gentle wash. In the larger egg follicles, above 6 mm dia, the nucleus was located peripherally just beneath the outer wall in the area which forms the center of the vascular anastamosis for the supply of the egg follicle. Thus after blood vessels were removed and the egg follicle washed it was easy to locate the nucleus (Fig. 12).

II. Isolation of the Nucleus

Different procedures were developed for each of three size classes of egg follicles, (A) 0.8 - 3 mm; (B) 4 - 8 mm and (C) 9 mm - 3 cm dia.

A. Egg Follicles of 0.8 - 3 mm

After determining the spot where the nucleus was embedded, the wall closer to the nucleus was held by a pair of fine forceps in such a manner that the pointed end of the forceps did not penetrate the membrana folliculi. Another pair of forceps was placed opposite the first forceps holding the fold. Both forceps were pulled in opposite directions. The intact oocyte covered with the membrana granulosa was

extruded as a semi-transparent sac containing clear fluid. The nucleus was easily located as a clear round ball in the translucent fluid. If the puncture was made too deeply a ribbon of nucleoplasm could be seen extruding in which the nucleus appeared as a transparent swelling.

B. Egg Follicles of 4 - 8 mm

The egg follicle contained a yellowish white yolk. A puncture was made away from the nuclear spot so as to allow most of the yolky fluid to be expelled. The egg follicle was transferred to another watchglass containing the 5:1 isolation fluid. The two forceps were slipped in through the puncture and the egg follicle pulled into two pieces taking care that the nucleus stayed in the center of one of the pieces. As the nucleus was facing upward, it was easily separated from the remainder of the materials in which it was embedded by a gentle movement of the forceps. It was transferred to a clean watchglass containing fresh 5:1 isolation medium.

C. Egg Follicles of 9 mm - 3 cm

The egg follicle full of yellow yolk was washed and placed on a piece of filter paper so that the position of the previously located nucleus was facing upward. A square or rectangular cut was made in the follicular membranes (vitelline and other theca) around the nuclear region. This cut was stepwise, first in the front; then on the sides, right and left; and lastly on the back of the nucleus (Fig. 17). The incized rectangular piece of the follicular membrane bearing the nucleus and some yolk was removed from the remainder of the egg yolk (Fig. 17) and immediately slipped onto a spatula and transferred to another watchglass containing fresh isolation medium.

This rectangular piece was then turned upside down. Thus the nucleus bearing surface of the rectangular piece was facing up and the follicular membranes down (Fig. 18). By a gentle movement of the forceps, the yellow yolk was removed leaving a circular bulging area around the nucleus which contained white of yolk. As the yellow yolk made the medium cloudy the cut piece was transferred to a watchglass containing clear 5:1 isolation medium, where the white of the yolk could be seen directly encapsulating the transparent circular area of the nucleus (Fig. 19). The follicular membranes supporting the lower half of the nucleus were separated from the remainder of the fibrous layers of the follicle (Fig. 19). At this stage the nucleus and its immediate covering of the white of the yolk and dispersed yellow of the yolk were on the follicular layers which from bottom to top were follicular epithelium, vitelline, and membrana radiata. The nucleus was then removed by removing the yellow and white of the yolk with either a tungsten needle or fine forceps.

The isolated nucleus (Fig. 20) was then transferred to another watchglass containing medium B of Callan and Lloyd (1960), (0.05 M-K/NaCl 7 parts, and 0.001 M-KH_2PO_4 3 parts with pH 6.4 - 6.6). Contained in the mixture was 0.5×10^{-4} M-$CaCl_2$ for isolation of lampbrush chromosomes (Gall 1966a). In transferring the nucleus with the blowing pipette (Appendix II) it was important to draw up a drop of the isolation medium before drawing up the nucleus, otherwise the nucleus often stuck to the dry wall of the pipette. The nucleus was cleaned by repeatedly drawing it in and out of the pipette. In a few cases, yolk plates and other materials of the endoplasmic reticulum were found sticking to the nuclear envelope. However, these

were removed by gently using the forceps.

III. Isolation of the Lampbrush Chromosomes

The lampbrush chromosomes were isolated from the nucleus in a special isolation chamber (Appendix II). A freshly isolated nucleus, obtained as previously described, was cleaned and transferred to the isolation chamber which contained medium B. While being observed through a stereoscope, the nuclear diameter was measured. With the aid of a pair of very fine jeweller's forceps a fine fold of nuclear wall was held tight and raised (Fig. 22). Later in a position near the forceps the base of the fold was held with a second pair of forceps. By pulling the two forceps apart with a slight downward motion the nuclear wall was torn open and the nuclear contents popped out, spreading on the floor of the chamber (Fig. 23). The slide containing the nucleus in the chamber was exposed to formalin fumes for 3 - 5 minutes. The inspection chamber was sealed and the slide was kept for the spreading of the lampbrush chromosomes.

A. Spreading

A drop of 1% acetic acid, made in full strength of commercial formalin was poured onto a water soaked filter paper kept in the moist chamber. The slide bearing the specimen was placed into the moist chamber and then the moist chamber was carefully placed into a refrigerator and left undisturbed for thirty minutes to four hours. After this period the chromosomes were well spread and fixed to the bottom of the chamber.

B. Fixation and Staining.

The coverslip or the slide, whichever was used for sealing the bottom of the isolation chamber, was separated from the well slide by means of a pointed scalpel. The specimen-bearing coverslip/slide was treated as a normal cytological slide and placed in a jar containing 5% formalin for one or two hours.

The coverslip/slide bearing the specimen was washed with distilled water. Two stains were used, Hematoxylin and Feulgen. For Hematoxylin staining, 2% ferric chloride was used as a mordent for fifteen minutes after which the slide was stained for one hour followed by two or three minutes washing in one percent ferric chloride solution. For Feulgen staining, ten minutes of 0.5 N hyrdrochloric acid was satisfactory to produce optimal hydrolysis of the chromosomal DNA.

Materials and Methods

Both ovarian tissue and isolated oocyte nuclei were used. Ovarian tissue was dissected from growing White Rock females from 1-22 weeks of age and from mature birds obtained from the University of Alberta Poultry Farm.

After the bird was killed and the ovary dissected, small blocks of the tissue were immediately placed into the fixative. Preliminary experiments indicated that Sanfelice's fixative mordant with 1% platinic chloride gave satisfactory results. After blocks were dehydrated in ethanol, they were embedded in paraffin and sectioned (7 - 10 U thick) by a hand operated Porter Blum Microtome. Sections were stained using Heidenhaine's Hematoxylin.

Preliminary experiments indicated that oocyte sections from mature birds contained lampbrush chromosomes. On the basis of this information later work on isolation was restricted to egg follicles from laying birds. Oocyte nuclei were isolated as outlined in Technique of Isolation of Lampbrush Chromosomes.

I. Autoradiography.

The procedure used for autoradiography was essentially that of Ebstein (1967).

Before isolation of the nucleus in 0.1 M (5:1) solution the well of the side was washed with 10 ug/ml Actinomycin D in water to reduce the background effect. A drop of A.D. H^3, 30 uc/ml solution in (5:1) B medium was placed into the inspection chamber in which the nucleus was to be transferred. The nuclear membrane was popped out with a pair of forceps. The lampbrush chromosomes were allowed to

spread for 2 - 3 hours at 0°C. During this time the slide bearing the isolated material was exposed to 50% ethanol to facilitate fixation and prevent sticking to the bottom of the coverslip. The A.D. H^3 solution was washed off by placing the slide into a 0.05 M solution of phosphate buffer, pH 6.8, for 5 minutes. Meanwhile the coverslip forming the base of the well was separated, washed in distilled water, and the sealing was removed by dipping into toluene and acetone solution. The coverslip was dipped into a subbing solution of 0.1% gelatin and 0.01% chrome alum and then air dried. The coverslip bearing the desired lampbrush chromosomes was coated with Kodak NTB-2 liquid emulsion and stored in a dark cabinet at 4°C. After a four-week exposure the preparation was developed for 3 min in D 19 at 20°C, stained with 2% Fastgreen at pH 2, and mounted.

II. Enzyme Treatment.

Using phase microscopy, studies of enzyme dissociation were conducted on the freshly isolated lampbrush chromosomes. The reports of Callan and MacGregor (1958), MacGregor and Callan (1962), Gall (1963), Trosko and Wolff (1965), Miller (1965), and Monneron (1966) contain information concerning the strengths of enzyme solutions and the corresponding pH to be used for enzyme treatment of lampbrush chromosomes. Isolation medium B was used as the carrier of the enzyme solutions. The enzymes, and their pH and concentration are given in Table 3.

Table 3. Enzyme, pH and concentration used in study of lampbrush chromosomes

Enzyme	pH	Concentration
Trypsin	6.2	0.25 mg/ml
Pronase	7.0	0.25 mg/ml
RNase	6.3	0.25 mg/ml
DNase	6.3	0.03 mg/ml

After the lampbrush chromosomes, isolated as earlier described, were well spread the top coverslip of the inspection chamber was removed. The slide was kept under the microscope, the well was rinsed twice with the isolation medium, and the isolation medium then removed.

The stage of an inverted Nikon microscope was fitted with a transparent plastic walled incubator. The enzyme solution was fed directly into the well of the side which contained the specimen. In this way handling of the slide between microscope and a conventional incubator located elsewhere was eliminated. Using a long drawn Pasteur pipette, feeding of additional enzyme solution into the well was steadily maintained.

III. Electron Microscopic Studies

Sections of isolated nuclei, isolated intact egg follicles of hens, ovaries from 7 week-old chicks, and whole mount preparations of isolated lampbrush chromosomes were studied under the electron microscope.

A. Isolated Nuclei.

To be sure of the presence of the nucleus, it was essential

that during isolation and subsequent treatment of fixation, dehydration, and embedding, the nucleus be kept under continuous examination under a zoom stereoscope. For fixing the nucleus during the process of isolation from the egg follicle, the isolation medium was slightly diluted with a drop of 2.5% glutraldehyde at pH 7.3. After isolation, either the nucleus was transferred to another watchglass containing fixative, or the nucleus was kept stationary under observation and a fresh quantity of fixative was added. After one hour the nucleus was washed with a phosphate buffer, pH 7.3, and counterfixed by exposing it to the fumes of 2% OsO_4 (Osmium tetraoxide) buffered at pH 7.3. The dehydration of the nucleus was performed by a series of ethanol. Epon mixture and other embedding media was added to the watchglass in which the nucleus was originally isolated.

B. Isolated Egg Follicles and Ovarian Tissue.

Ovarian tissue dissected from a 7 week-old fowl as well as from the intact egg follicles plucked from a mature bird's ovary was fixed, embedded, and sectioned. The procedure for both specimens was the same.

The birds were killed and immediately 2.5% glutaraldehyde solution at pH 7.3 was poured into the abdominal cavity thereby covering the entire ovary. The ovary was removed and sliced into small pieces which were placed into a fresh stock of the 5% glutaraldehyde fixative. The same procedure was repeated with egg follicles plucked from the mature ovary. After about 24 hours they were double-fixed in 2% OsO_4 solution, buffered at 7.3 pH for 12 hours. Later the tissue specimens were twice rinsed in distilled water and

dehydrated in a series of 50%, 70%, 80% and 90% ethanol for 10 minutes each and washed three times in absolute alcohol for 10 minutes. The tissue specimens were then dipped in propylene oxide for two consecutive periods of 5 minutes, and stored overnight in a 1:1 mixture of propyline oxide-Epon mixture. After all pieces of the specimen were placed into a separate gelatin capsule containing a fresh Epon mixture they were kept under vacuum for 15 minutes. After the air bubbles had disappeared the capsules were kept in a vacuum incubator at 60°C for 72 hours so as to polymerize the embedding mixture. The capsular gelatin was later removed and 0.1 U - 600 Å sections were cut and stained with uranyl acetate. These were then mounted on formvar-coated grids.

C. Whole Mount Preparations.

Freshly isolated nuclei were cleaned and washed. These were transferred onto 200 mesh formvar coated grids which had been placed in the bottom of the well of the inspection chamber containing isolation media. The nucleus was popped out onto the grid while being observed under a stereo-viewer. After the nucleus had been exposed to formaldehyde fumes for 3 minutes the inspection chamber was sealed. The chromosomes were spread on the grid by keeping the slide containing the grid in the inspection chamber in a fixed position. The slide was further checked before and during fixation and dehydration under an inverted phase microscope. After opening the inspection chamber by removing the top coverslip, the grids were picked up with curved forceps.

Whole mount preparations were prepared in one of three ways

using the wet grids: (1) Grids were transferred to an ethanol series and dried by evaporation in a vacuum. (2) Grids were washed and exposed to osmium fumes. Because the osmium fixative corroded the grids with dark stains, the grids were first exposed for 10 minutes to 1% neutral glutaralydehyde followed by a short exposure to 2% osmium fumes. The grids bearing the chromosomes were then passed through grades of ethanol for dehydration, and stained with 2% uranyl acetate for 10 minutes. (3) Other grids were dipped in 2% solution of phosphotungstic acid at pH 7 for 5 minutes. After dehydration the grids were observed under a Phillips 100 or 200 electron microscope.

Results

The results are divided into: (I) Light Microscopic Studies of Stained Sections, (II) Phase Microscopic Studies of Isolated Nuclei, and (III) Electron Microscopic Studies.

I. Light Microscopic Studies of Stained Sections.

For convenience the stained sections were divided into: (A) Growing Bird Oocytes and (B) Mature Bird Oocytes. Growing Bird Oocytes were further subdivided according to the age of the bird, and Mature Bird Oocytes were subdivided according to the size of the oocytes.

A. Growing Bird Oocytes

1. Oocytes, 7 Days Old. (10 - 70 μ):

An active proliferation was observed of the sub-epithelial cells which are later transformed to follicular cells (Fig. 24). All oocytes were located in the cortical region of the ovary. Some extra follicular oocytes which contained double nucleoli were observed (Fig. 25). Nuclei observed at this stage of small oocyte development were of irregular shape and size and in the majority of cases were eccentric and covered slightly more than half of the cytoplasm (Fig. 26). Chromosomal filaments which had an appearance of coiled ribbons beaded along their axes were scattered throughout the nucleus and often were touching the nuclear wall (Fig. 26). Therefore the nucleus has an appearance of a ball of ribbon as described by Loyez (1906). Occasionally, recently divided oocytes surrounded by cuboidal cells of tunica folliculi were noticed (Fig. 27).

A few of the 7 day oocytes were approximately 70 μ in diameter. Among the larger size of oocytes (Fig. 28) the nuclei were more regular

in shape and size than among the smaller oocytes. Chromosomes appeared to be separated from the nuclear wall. Some chromosomes were in a clear diplotenic configuration and continuous strings of chromosomes exhibited heavily stained granulations along their lengths. Presence of a single nucleolus with irregular boundary was also noticed (Fig. 28).

2. Oocytes, 14 - 16 Days Old. (20 - 143 μ):

The chromosomal morphology of the smaller oocytes of this age was similar to that observed in seven day old oocytes. Often one or two large nucleoli (3 - 4 μ) and diplotene chromosomes with condensed chromatin along their axes were observed (Fig. 30).

Large oocyte nuclei (Fig. 31) had distinctly enlarged chromosomal filaments. The chromatic granules were enlarged over those observed in small oocytes of the same age or in seven day old oocytes. Some chromosomes showing a small nucleolar like condensation at the cross of two bivalents (Fig. 31) were obviously in diplotene stage. Others which showed heavy granular condensation along their axes were also in diplotene stage. In larger oocytes the columnar epithelium of tunica folliculi was broader than in smaller oocytes (Fig. 31). The oocytes of the most common size, 50 - 70 μ, had a nuclear apparatus similar to that observed in larger sizes of 7 day old sections.

3. Oocytes, 18 Days Old. (20 - 170 μ):

Although most oocytes were still in the cortex of the ovary, they were beginning to migrate towards the medullary region. The morphological nature of the smaller oocyte nuclei was similar to that of the most commonly observed oocytes from 14 - 16 day old ovarian sections. However, in oocytes of most frequent size, the central chromosomes showed a polar

orientation and the peripheral chromosomes were parallel to the nuclear wall (Fig. 32).

A clear space, ectonucleoplasm, existed between the central zone of the nucleoplasm, which contained the chromosomes, and the nuclear envelope. Also, the nuclear wall became folded (Fig. 32). Although chromosomes in some oocytes were observed in typical diplotene shapes, most of the chromosomes showed a heavy chromomeric condensation along the axes, indicating that they were in the initial stage of barb formation. In larger oocytes, ranging from 148 - 160 U, the nuclei contained typical lampbrush chromosomes and a large nucleolus (Fig. 33).

This age, 18 days, was the earliest at which oocytes of 150 - 160 μ contained lampbrush chromosomes, although there were some indications of lampbrush formation in 50 μ oocytes of the same age (Fig. 32). Prior to lampbrush formation the number of nucleoli was variable but during lampbrush formation most cells contained a single large nucleolus (Fig. 33). Chromomeric condensations along the axes of chromosomes became faint as transverse striations (barbs) appeared on either side of the chromomeric points.

4. Oocytes, 3 - 4 Weeks Old. (20 - 143 μ):

The larger oocytes continued to migrate from the cortex into the ovarian medulla. Although smaller oocytes containing nuclei of early prophase and single nucleolus were seldom observed, oocytes identical to those of younger ages containing diplotene chromosomes were predominant. Some of the small oocytes contained reticulated nucleoplasm which was possibly resting nuceli (Fig. 34).

The majority of oocytes were approximately 50 μ which is considered by Brambell (1925) and Marza and Marza (1935) to be the Balbiani body stage. After the Balbiani stage, the cytoplasm increased to more than twice the size of the nucleus. The nucleoplasm consisted of two zones, the ecto- and endonucleoplasm and had a nuclear apparatus identical to that of slightly larger oocytes of a younger age; compare Fig. 35 with Fig. 32.

The larger chromosomes were arranged parallel to the circumference of the nucleus and were highly granulated. Their chromomeric boundaries became expanded and diffused (Fig. 36). Although in the largest oocytes the chromosomes were scattered in different directions, near the periphery of the endonucleoplasm they tended to have a polar arrangement (Fig. 37). Because the chromosomes at the periphery were parallel to the circumference of the nucleus and the central chromosomes were unorganized, it may be that the movement of the chromosomes was limited by the ectonucleoplasm. The areas of heavy granulation were the first to have barb fibers on either sides of the chromosomal axis and it may be that the peripheral chromosomes were the first to initiate barb formation.

The lampbrush chromosomes were easily identified in oocytes of 70 μ (Fig. 36) and larger. This size of nucleus containing lampbrush chromosomes was characterized by the nuclear boundary becoming rough, corrugated and doubly folded.

5. Oocytes, 4 - 5 Weeks Old. (35 - 183 μ):

The smallest oocytes were about 35 μ, of which a majority had a large single nucleolus. Most of these small oocytes were at the Balbiani

stage with the nucleus showing chromatic reticulum (Fig. 38) and having the appearance of a typical resting nucleus. The most commonly found oocytes(about 50 - 100 μ) contained active nuclei with diplotene bivalents lying parallel to the circumference and were associated with a Balbiani body towards one end of the nucleus (Fig. 39). The larger oocytes ranged from 400 - 500 μ in diameter with nuclei about 120 μ diameter.

6. Oocytes, 6 - 7 Weeks Old. (30 - 600 μ):

The appearance of nuclei of the smaller oocytes was almost identical to those observed in 7 day old oocytes. In some oocytes an unbalanced ratio of nucleus area to cytoplasm area plus the morphology of the follicular wall indicated some oocytes were approaching to atricia (Fig. 40). In a majority of the smaller oocytes the cytoplasm was actively engaged in Balbiani body formation or in synthetic activity (Fig. 41).

The intermediate sized oocytes contained chromosomes which in the center were in the process of becoming orientated and near the periphery were already orientated and showed a distinct polarized arrangement (Fig. 42).

7. Oocytes, 9 - 10 Weeks Old. (30 - 800 μ):

The smallest oocytes were 30 - 42 μ in diameter. However, the majority of the observed oocytes were between 200 - 600 μ in diameter and the largest oocyte of this age was about 800 U. In oocytes of approximately 500 μ the nucleoplasm had a globular appearance. Not only did the nucleoplasm become globular but also vacuolated to the extent that the fibrillar structures of chromosomes and their axes were

hard to locate. However, in a number of larger oocyte nuclei, lampbrush chromosomes were observed.

8. Oocytes, 12 - 14 Weeks Old. (31 - 800 μ):

In nuclei of larger oocytes the dispersed granules increased in size and density. At this stage the size and intensity of nucleoplasmic granulation of a 400 μ oocyte (Fig. 43) was similar to that of a 500 μ oocyte from a 7 week old chick (Fig. 44). Therefore a larger oocyte (larger nucleus) of a younger age (7 week) gives better resolution of lampbrush chromosomes than a 12-14 week old oocyte. This can be seen in Figs. 44 and 45 where the chromosomes are located parallel along the periphery of the nuclei and show distinct lateral loops at the chromomeric regions along their axes.

The smallest oocytes which contained lampbrush chromosomes showed distinct axes which appeared to be discontinuous at the interchromomeric regions. In some nuclei (Fig. 43) chromomeres appeared to be split longitudinally along the axes of the chromosomes. The axes of lampbrush chromosome loops which appeared to be spun out from the sides of the corresponding chromomeres were granulated in a single file. The loop granules were similar in density, dimension and staining behaviour to the peripheral granules of the nuclei (Figs. 46 and 47). However, the granules located at the base of each loop were larger than those at the apex.

9. Oocytes, 16 Weeks Old. (35 μ - 1.8 mm):

The minimum size of oocytes observed was approximately 35 μ with 16 μ nuclei while the maximum size of oocytes was about 1800 μ

with a 250 μ nucleus. The large egg follicles containing such oocytes could be visually located on the ridges of the freshly dissected ovaries (Figs. 14 and 15). Because the nuclei of such large oocytes were so highly granulated and vacuolated, efforts to identify the chromosomal filaments in the nucleoplasm were terminated.

B. Mature Bird Oocytes.

For convenience, the oocytes of mature birds were arbitrarily grouped into: (1) 30 - 100 μ, (2) 100 - 500 μ, (3) 500 - 1000 μ, (4) 1 - 2 mm, and (5) larger than 2 mm.

1. Oocytes, (30 - 100 μ):

The small size oocytes (Fig. 48) contained eccentric nuclei with some chromosomes showing zygotene configurations and other chromosomes showing condensed, large, heavily stained chromomeres. In general, chromosomes from oocyte nuclei of mature fowl had a heavier Hematoxylin stain than those of the same size oocyte from younger ages. The absence of a nucleolus and the presence of granular or condensed chromosomal axes were consistently observed in nuclei from oocytes of this group. Because some of these oocytes also showed incomplete double chromosomal filaments (Fig. 48) which were similar to those described in one day old chick oocytes by Hollander (1904), they were obviously either dormant or resting.

Large oocytes, 65 μ dia, contained nuclei, 25 μ, still accentrically located which appeared to be associated with the Balbiani body (Fig. 49); suggesting that both the nucleus and the Balbiani body were engaged in synthetic processes. Association of the folded nuclear wall with the Balbiani body is vividly shown in Fig. 49. The

chromosomes were beginning to be either arranged in a polar manner or parallel to the periphery of the nucleus. In these larger oocytes the chromomeres were more heterochromatic and diffused, indicating an initiation of barb formation.

2. Oocytes, (100 - 500 μ):

In oocytes of larger sizes (Fig. 50) the nuclear wall became folded and the ecto- and endonucleoplasm well defined. The smallest oocytes that showed distinct lampbrush chromosomes were about 143 μ with 44 μ nuclei (Fig. 50). In oocytes up to 500 μ the lampbrush chromosomes were oriented parallel to the circumference of the nucleus. A nucleolus was not uncommon in nuclei of this range of oocytes. In mature birds, oocytes of 150 μ and larger contained lampbrush chromosomes with very clear chromomeric condensations containing distinct lateral loops as radiating bristles (Fig. 50).

3. Oocytes, (500 μ - 1 mm):

Oocytes from 500 μ to 1 mm contained nuclei up to 200 μ (Figs. 52 and 53) and a single nucleolus. The chromosomes were in the lampbrush stage and were quite distinct. Although Fig. 51 only shows half of the nuclear field, 12 lampbrush chromosomes were counted in this particular nucleus. In most oocytes the chromosomes were generally parallel to the circumference of the nucleus although in some oocytes there was no orderly arrangement of the chromosomes. It is very interesting to note that the nuclei shown in Figs. 51 and 53 are almost identical with the results as described by Hollander (1904) for twenty day old chicks and by the author for eighteen day old chick oocytes.

4. Oocytes, (1 - 2 mm):

Exact location of the nucleus was not fixed within the oocyte boundary. The nucleoplasmic granules, probably the peripheral nucleoli, had increased in size and number such that the recognition of lampbrush chromosomes was difficult (Figs. 54, 55, and 56). However, in slightly larger ooctyes (1.8 mm) stained with Hematoxylin (Figs. 57 and 58) clearly outlined lampbrush chromosomes were observed.

5. Oocytes, (Above 2 mm):

The stage of development of oocytes above 2 mm is referred in the literature as the second phase of active growth of oocytes. In these larger oocytes (4.5 mm) all the follicular layers were extensively enlarged and the vitelline membrane became well differentiated between the membrana radiata and membrana folliculi.

Continuity of chromosomal filaments was observed in an oocyte up to 4.6 mm dia containing a nucleus about 0.3 mm (Fig. 59). Some area of the chromosomes appeared as knotted nodes and the nucleoplasm was filled with larger knot-like rounded bodies which may be peripheral nucleoli. As the oocytes grew larger, the nucleoplasm became more vacuolated and granulated (Fig. 59).

C. Changes in Oocyte Parameters.

The measurements of the egg follicle, oocytes, and corresponding nuclei of the stained slides of growing and mature birds observed in the chromosome study were grouped. In each age, minimum and maximum diameter of the oocytes and the depth of follicular epithelium range of oocytes based on a mean of 5 sections (measurements of nucleus and

Table 4. Diameter of nucleus and thickness of follicular epithelium arranged according to diameter of oocyte and age (stained section preparations)

Oocyte diameter (microns)			AGE											
			1-2 weeks		2-4 weeks		4-8 weeks		8-12 weeks		12-16 weeks		22 weeks	
min		max	min	max	min	max	min	max	min	max	min	max	min	max
10	-	20	7/4*	11/9	8/6	11/10								
21	-	40	12/4	18/10	16/8	22/12	16/7	24/9	15/7	23/10	16/6	25/9	14/10	20/**
41	-	60	16/6	20/19	20/10	27/12	17/10	27/11	20/7	28/12	21/7	27/9	20/11	27/15
61	-	90	**	40/18	27/12	32/16	32/9	42/12	30/7	38/12	34/7	40/11	28/9	55/14
91	-	150	**	**	33/8	50/14	34/8	62/10	45/9	68/20	42/9	70/18	49/6	67/15
151	-	300			**	57/14	50/9	68/12	60/9	80/13	68/10	83/16	90/11	**
301	-	500					78/8	123/15	80/10	100/14	**	125/10	153/10	200/17
501	-	1000					**	**	**	**	**	200/9	**	250/19
1001	-	2000									**	250/15	**	285/17

*Nucleus diameter/thickness of follicular epithelium.

**Not recorded.

follicular epithelium) are given in Table 4. Mean oocyte diameter and nuclear diameter for different size egg follicles of laying birds are presented in Table 5.

Table 5. Diameter of nucleus and oocyte for different size of isolated egg follicles of laying hen (stained section preparations)

Egg follicles diameter (mm)	Number of observations	Oocytes* diameter (mm)	Nuclear* diameter (U)
1.3	2	1.0	200
1.9	2	1.2	120
2.0	5	1.6	284
3.0	5	2.8	200
3.5	1	2.8	255
4.5	1	4.1	250
5.0	1	4.6	300
5.6	1	5.0	233
6.5	1	6.1	261

* mean value

II. Phase Microscopic Studies of Isolated Nuclei.

Results are presented in three sections: (A) Morphology of Isolated Chromosomes, (B) Effect of Enzymes, and (C) Autoradiography.

A. Morphology of Isolated Chromosomes.

Initial efforts to study morphology of lampbrush chromosomes in the isolated nuclei involved placing a coverslip over a freshly isolated nucleus. Nuclei from smaller egg follicles had relatively brighter boundaries and two or three diplotene chromosomes could be resolved (Figs. 60 and 62), however this was not true for nuclei from larger egg follicles (Figs. 63, 64 and 65).

In intact vesicles, because of the nuclear membrane, the nuclear contents of larger egg follicles were difficult to observe. A marked improvement in the resolution of chromosome morphology was obtained by the removal of the nuclear wall, compare Fig. 61 with Fig. 60. Isolation of lampbrush chromosomes from egg follicles, 0.6 - 30 mm, was as described in the Technique of Isolation of Lampbrush Chromosomes (Chapter 2). For convenience the description of isolated chromosomes is categorized into : (1) Lampbrush Chromosome Bivalents, (2) Lampbrush Chromosome Elements , and (3) Nucleolar Lampbrush Chromosomes.

1. Lampbrush Chromosome Bivalents.

Frequently nuclei from younger follicles (up to 2 mm) yielded chromosomes with typical characteristics of diplotenic bivalents and

these chromosomes in many cases had small stumpy lateral loops (Figs. 66, 67, and 69). The homologues were associated in at least 4 or 5 different places suggesting the crosses in the bivalents may be involved in chiasmata formation.

However, the presence of a large nucleolus in nuclei isolated from egg follicles up to 2.5 mm does not eliminate the possibility that the morphological status of some chromosomes may be in an early prophase (Fig. 68). The terminal end of these bivalents appeared to be splitting into two sister chromatids (Figs. 67, 68 and 69). Presence of chromatic granules and spheres characterized them as late diplotene or lampbrush chromosomes. Chromosomes in this particular configuration were never observed in nuclei isolated from egg follicles larger than 3 mm. Also, follicles less than 3 mm had one, two, or three bivalents in the classical diplotene configuration (Figs. 66, 67, 69, and 70). This may be considered as evidence of the asynchronous breakdown of the diplotene bivalents.

The two arms shown in all these chromosomes were obviously not sister chromatids but rather homologous bivalents. The radiating loops appeared to be shorter than those described for amphibian lampbrush chromosomes.

The chromosomes shown in Figs. 67 and 68 were procured from two 1 - 1.5 mm egg follicles. These chromosomes were about 40 μ long. The two bivalents were involved in crossing at points A, B, C, and D suggesting 4 chiasmata formations. Chiasma D appears to be centric. The bivalents tended to separate at chiasma C. Chromomeres in these chromosomes were of variable shapes and sized and exhibited different

optical densities. The chromomeres with stumpy projected loops were lighter and smaller and perhaps metabolically more active. The chromomeres were spherical, angular, and not perfectly rounded. They were broader (greater in diameter) than the width of the monovalents. Although the chromomeric boundaries were not precisely defined enough to give an accurate measurement, chromomeres measured about 2000 - 3000 Å. The width of the monovalent (chromonema plus matrix) was less than 1 μ with a minimum of 3428 Å. Therefore, each chromatid would be at least 1500 Å. In these chromosomes the denser chromomeres were about 1 μ while the smaller and lighter ones were beyond the limit of microscope resolution. The larger chromomeres were probably uncoiled and ready for extending the loops.

Similar chromosomes were observed from other 1 mm egg follicles (Figs. 66 and 69) and were approximately the same length as the ones described above. The crosses at B and C resembled the former ones except the very well defined denser chromomeres at chiasma C (Fig. 66) appeared to be involved in exchanges by radiating processes. Apparently a section of nucleus passing from such a chromomere will resolve an aster-like object in the nucleus with 4 radial projections as has been shown earlier in the light microscopic study of stained sections. Other chromatic granules on the right arm of Fig. 69 appeared similar in position to those of Fig. 67. The chiasmata at A and B in Figs. 66, 67, and 69 appeared chromomeric.

Chromosomes procured from slightly larger egg follicles (Figs. 69 and 70) showed larger radiating loops which originated bilaterally from the thick beaded spots (chromomeres) arranged linearly on the main axes of the chromosomes. Also the chromosomes carried chromatic

granules (chromatic nucleoli). These chromosomes were considered as typical lampbrush chromosomes. Chromomeres were identified and chromonema was evident at places. Just as in nuclei isolated from the smaller egg follicles the lateral loops in these cases were not as large as is described in amphibian lampbrush chromosomes. The lateral loops (Figs. 70, 72A, 74, and 75) were also beaded at intervals and formed a single row on the loop axes. Also these lateral loops carried chromatic granules (chromatic nucleoli) of similar dimension and density as those embedded on the main axes of chromosomes (Figs. 74 and 75). Koltzoff (1938), from studies of stained sections, proposed the beads on lateral loops as secondary chromomeres and that in this stage of oocyte the loops studded with such chromomeres were in the process of retraction.

On some loops large chromatic granules were easily identified which corresponded to the findings obtained from the stained section study of growing and mature birds oocytes. It was also observed that at a certain stage the two homologues of the bivalent along with their lateral loops separate (Fig. 73). However, it is difficult to say if it was a sister chromatid or the whole chromatid (monovalent).

Such diplotenic chromosomes were restricted to smaller egg follicles up to 3 mm in which each nucleus at a time yielded a maximum of 3 - 4 chromosomes which appeared as lampbrush stage of macrochromosomes. However, the remainder of the chromosomal complement isolated from the nuclei of small as well as of large size egg follicles was a chromomeric fibrous structure of heteromorphic nature and could be regarded as a series of transitional stages of chromosomes which covered the post diplotenic, resting and diakinetic phases of nuclei.

2. Lampbrush Chromsome Elements.

a. Lumpy loop elements. The lampbrush chromosome elements of this type were common constituents of younger egg follicles (Figs. 76, 77, 78, and 79). At places the axes of chromosomal strands bearing chromomeres were well defined but at the extremities merged into the matrix material (Figs. 76 and 77). The lateral loops appeared to be a series of unorderly branched structures radiating from the axis of the main strand. These lumpy loop elements did not spread well even after having been kept for 24 hours in the well slide. However, the Brownian movements they exhibited suggested that the matrix material of the nucleus may be involved with lateral loops of the lampbrush chromosomal fibers. When freshly isolated the nuclei yielded fibrous structures of two different densities, one was denser, thicker and more refractible, about 3000 Å wide, and the other was thinner, finer and diffused and was beyond the limit of resolution (Figs. 78 and 79).

The thicker fibres appeared similar to the loop bridges (Figs. 78 and 79) which are usually present in one but rarely in both chromonema of amphibian lampbrush chromosomes (Srivastava and Bhatnager 1962). However, they were obviously not loop bridges because the axes of the fibers showed chromomeric beads and chromatic granules and further associated with the axes were lateral loops which were very fine and undulating. This suggests that the thin chromosomal fibers may have appeared as a result of uncoiling of the thicker elements (chromatids, Fig. 77) and at the light microscope level they formed the ultimate fiber of the chromosome (chromonema). The lateral loops were

very fine, long, undulating, and had similar dimension and morphology to the finer fibers (chromonema).

b. Mulberry type elements. These were the most common chromosomal elements of nuclei isolated from 2 - 9 mm egg follicles (Figs. 80 and 80A). When the nucleus was opened the chromosomal elements adhered to the nuclear wall and appeared like fragments of rope. Because each element and undulating axes bent at intervals into different directions (Figs. 80, 80A, 82, and 82A) and on the axes they carried lateral beads or stumps (Fig. 81A) instead of large loops they were referred to as Mulberry type elements of the lampbrush chromosomes. Some of the elements had beaded lateral branches (Fig. 81).

Generally mulberry type elements were of two types: (i) small and stumpy up to 3 μ long (Fig. 83) and (ii) large and slender. Even after spreading in the slide well, under high magnification the smaller elements still appeared as a typical mulberry-like structure (Fig. 81A). Also some of these smaller elements formed chiasma-like crosses (Fig. 82A) and had a bivalent chromosomal appearance (Fig. 82). Their axes were ornamented with chromomeres and chromatic granules which indicate a probable lampbrush stage of the micro-chromosomes.

The larger chromosomal elements as shown in Figs. 84, 85, and 86 ranged up to a length of 48 μ and had straight axes with linear chromomeres equidistance apart. The straight axes were studded with chromatic granules, generally at the extremities. As the depth and dimension of the lateral loops were the same as the main axes it was difficult to visualize the quarternary structure of the chromosome. After keeping for 4 - 6 hours in a sealed inspection chamber containing

B medium these chromosomes become well-spread (Figs. 84, 85, and 86). Therefore the limit of the basic chromosomal strands (chromonema) and lateral branches of the same width along the axes measured was about 2000 Å.

In all cases (Figs. 84, 85, and 86) the axes and the side branches or lateral loops were of the same thickness and had a similar chromomeric pattern along the entire length of the axes. Chromomeres on the axes measured from 0.2 - 1 μ in diameter.

Examination of these chromosomal elements (Figs. 84, 85, and 86) indicated that the basic light microscopic fibres (2000 Å) consisted of two basic strings which were coiled around each other along the entire length of the chromosomal structure.

On the extreme right of Fig. 85 a bivalent bridge as described by Srivastava and Bhatenagar (1962) may be seen. However, this bivalent bridge may not be considered due to a single chromonema. Each of these strings, regardless of how fine they became after spreading, displayed a similar chromomeric pattern. The results shown in Figs. 84, 85, and 86 suggest that the interchromomeric fibers (chromonemata) may have been so fine that they were beyond the limit of resolution. The chromonema also carried attached granules which varied in size from 0.5 to 1.5 mm diameter (Fig. 85) and could be seen in doublets (Figs. 85 and 86). The least diameter of the chromomeres observed was around 3000 Å.

3. Nucleolar Lampbrush Chromosomes.

During active yolk synthesis the lampbrush chromosomes isolated from the nucleoli of larger egg follicles were often characterized by the presence of several rounded bodies, spheres and chromatic granules

henceforth called nucleoli along their axes. A majority of these rounded bodies were refractile, solid and spheroid in nature.

The following types of such elements were generally observed from egg follicles of size order up to 3 mm and beyond 5 mm diameter:

a. A class of chromosomal elements with fine straight or branched axis of about 2000 Å wide and linearly arranged chomomeres (Figs. 87 and 88). Along the axis were attached chromatic nucleoli which were solid, dense, and circular. All these attached nucleoli had a similar dimension of about 2 μ diameter.

b. Elements with branched and lumpy looped strands or ribbon-like axes, bearing dense refractile nucleoli of variable sizes, 0.8 - 5.5 μ diameter (Fig. 89). In stained preparations nucleoli took as heavy Hematoxylin and Fast green stains as the axes of chromosomes (Fig. 90).

c. Typical nucleolar lampbrush chromosomes which were identified and classified separately because: (i) they were identical to commonly described lampbrush chromosomes of amphibians (Dodson 1948, Gall 1954a, and Wischnitzer 1961), and (ii) the chromatic nucleoli and spheres attached to the axes of the chromosomes were of variable size.

Because of the presence of numerous granules and spheres of varying sizes attached along the axes, the boundaries of lampbrush chromosomes (Figs. 105 and 105A) were fuzzy. The larger chromatic granules (chromatic nucleoli), about 5 μ, were located in the middle of chromosomes with smaller granules (0.7 μ) localized along the length of the chromosomes but more predominant at the terminals.

The oocytes which contained such chromosomes are contained in egg follicles in second or third phase of rapid growth in fowl oocytes.

These chromosomes measured about 50 - 60 μ in length. Their axes where they appeared free between attached nucleoli (chromonemata) measured, by light microscope, about 2000 Å thick (Figs. 105 and 105A). The lateral loops appeared stumps and not well extended. However, on the basis of morphological appearance and the results of enzyme action and autoradiography such chromosomes were designated a typical nucleolar lampbrush chromosome. Such a configuration of lampbrush chromsomes may have appeared by separation of one of the homologues from the bivalent chromosome. The nucleoli attached on the axis of such lampbrush chromosomes are discussed in the next chapter.

B. Effect of Enzymes.

1. Trypsin.

The first observable action after the application of trypsin was the fuzziness of the boundary of the chromosome. After a few minutes (5 - 10) of enzyme action the matrix appeared to be dissolving and detaching from the main axis and from loops. Chromatic grains and granules started detatching and moving away from the loops and from the axes of the chromosomes where Brownian movement was observed (Figs. 85 and 91). In most cases, all chromosomal elements became fuzzy and cloudy immediately after adding proteolytic enzyme solution. Later the fibers became thinner. After washing with fresh isolation medium the chromosome elements became optically brighter. Although the chromosomes were watched for 24 hours no detectable change in chromosomes was observed (Fig. 91). The addition of a small quantity of fresh crystalline trypsin caused general contraction of the long axis (Fig. 92).

2. Pronase.

The action spectrum of pronase is very large (Monneron 1966). In the present experiment it was observed, that compared to trypsin, pronase had a very drastic action. In higher concentrations (0.5 - 1%) its action was so violent as to suggest a DNase contamination. After only 3.5 min exposure of the chromosomal elements to pronase (Fig. 93) the main axis and the side loops had not only become detached from the matrix material but also became finer, and less bright (Fig. 94). The results suggest that pronase helped in uncoiling and spreading the entire fibrillar structure of the lampbrush chromosome. Therefore, one may assume that the chromosomal axes and loops after treating with pronase still consist of DNA, RNA and some histones; or pronase-resistant proteins. The pronase treated fibers were about 2000 - 4000 Å thick which is the same thickness as obtained for freshly isolated lampbrush chromosomes and their elements after they were completely spread in the isolation media. There was no breakage or dissociation (at least at the microscopic level) of the fibrillar elements of the lampbrush chromosomes by a milder concentration of pronase. However, a higher concentration of pronase solution, which was observed to be more violent in action, may accomplish clearing of chromosomal fibers to the submicroscopic level leaving only fibers with DNA, RNA, and some pronase resistant materials. (Monnezon, 1966)

3. DNase.

Four samples of heteromorphic lampbrush chromosomal elements as described earlier (Figs. 95, 97, 99, and 102) were isolated. The well slides containing such elements were treated first with pronase

solution (Fig. 100) and later with DNase.

Washing with a pronase solution caused such elements to react with DNase because within 10 minutes the long central chromomeric axes as well as the lateral loops in most of them started disassociating linearly. A half hour after a fresh amount of DNase solution was added all elements were broken into pieces which were transverse to the axes (Figs. 96, 98, 101, and 103). In a few cases RNase solution helped in dissassociating chromatic granules from the axes of chromosomes (Fig. 104). Four additional samples of isolated lampbrush chromosomes (Figs. 105, 108, 110, and 112) were subjected to DNase solutions for variable times. A lampbrush chromosome (Fig. 105) was treated for 5 hours without any visual break (Fig. 106). However, immediately after adding two drops of pronase or trypsin into the inspection chamber which already contained the chromosomes and DNase solution, the long axis of the chromosome started breaking and within 4 min a complete dissociation of axial fiber along the lateral loops, including detachment of nucleoli and matrix material, was observed (Figs. 107, 109, 111, and 113). Obvious reduction in size of chromatic nucleoli was observed (Figs. 107 and 113) and beaded ring nucleoli (Fig. 105A) appeared disintegrated (Fig. 107A). These results indicated that for microscopic breakage of chromosomal material, simultaneous contact of DNase and pronase was needed.

C. Autoradiography.

Additional evidence that the elements originated from the lampbrush chromosomes was obtained by autoradiography.

1. Some of the common lampbrush chromosomal elements were

exposed to tritiated Actinomycin D. After 4 weeks of exposure the large chromosomal elements from a 400 µ nucleus 2.5 mm egg follicle (Fig. 114) as well as small mulberry type elements isolated from a 4 mm egg follicle (Fig. 115) contained labelling.

2. Lack of a stage micrometer on the inverted phase microscope prevented an accurate location and identification of areas on the treated/processed slides. To avoid uncertainty of location either the content of a freshly isolated nucleus (mass of unspread chromosomal elements) (Fig. 116) or those preparations having a marker (Fig. 118) were fed with tritiated Actinomycin D. The identifiable features were identical before and after the slides were processed (Figs. 117 and 119). All the nuclear elements excluding chromatic granules and nucleoli were labelled.

3. Further Mulberry type elements (Fig. 120) and nucleolar lampbrush chromosomes (Fig. 122) were identified and spread after isolation from 400 µ nuclei contained in 6 mm and 2.5 mm egg follicles, respectively. They were fed with tritiated Actinomycin D. After 4 weeks exposure the processed slide showed labelling of chromosomes. However, chromatic nucleoli were not labelled (Figs. 121 and 123).

III. Electron Microscopic Studies.

Two techniques were utilized for electron microscope studies of lampbrush chromosomes, namely, (A), Whole mount preparations and (B) Sections.

A. Whole Mount Preparations.

Stained preparations from 360 U nuclei of 3 mm egg follicles contained mulberry type chromosomal elements which retained their

individuality when viewed under low magnification (Fig. 124). The nucleoplasmic material still surrounded each chromosomal element which appeared to be composed of a twisting, winding, branched framework of two basic strings (Figs. 126 and 127), varying in width from 250 - 300Å. These fibers were irregularly folded and varied in diameter along the length.

An analogy to the loop matrix proposed by Miller (1965) was observed in these chromosomal particles (Figs. 126 and 127). On the loop material and basic chromosomal fibers the appearance of the variable types of matrix material suggests a scattered or cluster of ribosome-like particles. The basic chromosomal fibers were exposed at places which provided evidence of a long continuous fiber along the entire network. The basic granular unit of matrix material ranged from the smallest, 300Å, (Fig. 127) to the largest, 400Å, (Fig. 126) and appeared as ribosomal particles.

Nuclei were also opened directly on to grids placed into 2% phosphotungstic acid solution (PTA). The chromosomal material contained large particles of fibrous masses (Fig. 125) with fibers measuring about 250Å wide. However, when the chromosomal elements isolated from a 3 mm egg follicle nucleus were spread on to the grid and were not stained by 2% PTA (Figs. 124, 128, and 129) they were found to be independent units of organized branched systems with a dichotonomy in optical densities of fibers. The chromosomal elements having lower density and finer fibers (about 200Å) were well spread and uncoiled whereas in the other fiber-system the basic chromosomal fibers (Figs. 128 and 129) were similar in coiling pattern to that observed in the chromosomal fibers from interphase nuclei of Triturus (Wolfe and Grim 1967 and Ris 1966).

Close examination of these basic fibers, which formed a branched network of coiled ribbon-like string, revealed they were also approximately 250Å dia. Although not detected, there was most likely a central point from which the branching had radiated.

Chromosomal materials were also obtained from 1.7 mm egg follicle nuclei. The whole mount preparation after dehydration was stained with 2% Uranyl acetate. Micrographs of some chromosomal particles which appear to be actively engaged in nucleoli formation, or appeared as nucleoli bearing lampbrush chromosomes (Figs. 130 and 133) measured about 250Å. Therefore the main strand of fiber which appeared to be beaded or coiled along its entire length measured 600 - 700Å. The bead like spherical particles in the matrix material along the entire loop of fiber ranged from 600Å to 2 μ and most of them appear to be smaller chromatic nucleoli. The main strand, observed attached to larger nucleoli by means of two basic chromosomal fibers which were about 200 - 250 Å in dia (Figs. 132 and 133), is evidence for the strand being bipartite.

A differentiation in optical density as well as thickness of the chromosomal fibers suggests that the finer fibers of 200 - 250 Å dia were the basic fibers, microfibrils, and that two of these may compose the main strand of the chromosome which measured about 600 - 800 Å in diameter (Figs. 130 and 133). Although additional confirmation is needed that the basic fibers of 250 Å are the main strands of the barb chromosomes which have completely uncoiled and were spread, Wagenaar and Mazia (1969) found a similar pattern of chromosomal fibers when they studied spread meiotic chromosomes of the sea urchin.

B. Sections.

1. Isolated Nuclei.

Micrographs of the oocyte nuclei indicated a reticulated network of branched fibers, localised with other matrix material, close to the nuclear walls (Fig. 134). The reticular fibers were never smaller than 100 Å nor more than 250 Å thick. They appeared to originate as lateral extensions from either the chromomeres or from the area which formed the central axis. A main axis and side loop was observed (Fig. 134) which suggested a twisted chromosomal axis having an array of chromomeres in which each chromomere covered .5 μ area of condensed fibrillation. However, the fibers appeared associated with dense spherical particles of 300 - 400 Å, probably ribosome-like particles which were connected in chain or aggregated mass.

Attached to the nuclear wall were a number of condensed fibrillar elements which may have been due to a polar arrangement of chromosomes or were chromomeres displaced due to nuclear shrinkage by direct fixation of the isolated nuclei. However, no satisfactory observation concerning gross morphology of the lampbrush chromosomes could be made among the sectioned material studied.

2. Isolated Egg Follicles.

Electron micrographs were made of 0.1 μ and 600 Å thick sections of 2.3 mm egg follicles stained by 2% - 8% Uranyl acetate. No blebbing of nuclear wall was observed. The entire nucleoplasm appeared to be covered with a reticulum of chromosomal microfibrils and therefore it was hard to resolve the structural boundary of lampbrush chromosomes. The basic fibers of the reticulated network consisted of two strings of

microfibrils, each 150 - 200 Å dia. These two strings were parallel and were spread in all directions, and had periodic constrictions and expansions. This biphasic morphology of the two fibers caused circular vesicular areas (microvesicles of the nucleus) to appear along their entire length (Figs. 135 to 139). Individual microfibrils appeared to bear dense particles of ribosomal nature (Figs. 135, 136, and 138).

The entire nucleus was a network of branching microfibrils intercepted by microvesicles and trabeculae-like gaps produced by the absence of the fibers.

3. Ovarian Tissue.

Conventional electron microscopy procedure was followed. Earlier results had indicated that the lampbrush chromosomes could be easily observed in oocytes of 7 week old fowl. In smaller oocyte nuclei (Figs. 140 and 141) it was easy to observe the chromosomes in cross section with their radiating fibrous boundaries. By careful examination of the long axis, the presence of chromomeres could be determined. In bigger oocytes and under higher magnification (Fig. 142), the chromatin microfibrils appear to be dispersed throughout the entire nucleoplasm. The chromosomal boundaries of the axis and short lateral loops were observed in larger oocytes under low magnification (Fig. 143). The lateral loop-like projections of the chromosomes, which measured roughly 800 Å in dia, were detectable. It appeared that the axis had rows of transverse barbs of the same dimensions. Although continuity of the long axis of chromosomes connecting the chromomeres was not clear (Figs. 141 and 143) the photographs suggest that the main strand of the chromosome was in two longitudinal cores of fibers which were sectioned in their twisted configuration.

Discussion

I. Stained Sections.

The present study suggests that during the process of oogenesis the ovarian tissue of the fowl remains active for a long period of time. For example, proliferation of sub-epithelial cells apparently involved in the formation of follicular layers of cubical cells suggested encapsulation of extrafollicular oocytes occurred in 7 day old ovaries. Double oocytes enclosed by a single common layer of follicular epithelium were also observed in ovaries up to 2 weeks of age. Furthermore, up to 4 weeks of age, an active migration of larger oocytes continued towards medullary regions of the ovary.

Also chromosome transformation from diplotene to lampbrush chromosomes occurred as early as 18 days and continued until maturity. The sudden increase by the 25th day, in the number of relatively large oocytes ranging from a low of 20 μ to a maximum of 200 μ dia are all points indicating a dynamic population of oocytes and corresponding active nuclei. This disagrees with Goldsmith's (1928) observation of oocyte nuclei resting from the 2nd day until the 65th day.

Goldsmith's observation that chromosomes are in a resting stage from the 2nd day to the 65th day needs clarification. If he was referring to the smallest oocytes during this period then his results would agree with those obtained in this study, but if he was referring to the larger or more predominant oocytes of this age, then his results would not agree with the results obtained here. The concept of a resting oocyte has to be considered as proposed by Durme (1914) rather than Goldsmith, namely, that it represents a temporary pause or cessation in growth and

activity.

The growth of oocytes immediately after they become intrafollicular (4th day after hatching) is asynchronous (Romanoff, 1960). At maturity, some of the oocytes must be larger and more mature than the other oocytes because of the nature of egg production of domestic fowl. It is accepted that nuclear activity is related to cytoplasmic activity, therefore the smallest size of oocyte at a definite age must contain a less active nucleus than larger oocytes. However, in this study no definite pattern concerning specific nuclear apparatus, i.e. chromosomal structure forming pseudoreticulum could be observed.

Irrespective of the stage of oogenesis, the activity of oocyte and its nucleus may be stopped because of a restricted blood supply. Nevertheless, the presence of a large nucleolus indicates that because of the stored RNA in the nucleolus the oocyte will remain active, at least for a short period. In nuclei of the smallest size of oocytes, condensation of chromatin along the axes of the chromosomes was commonly observed which would suggest that these oocytes and nuclei were in a temporary pause prior to further development. This observation differs from Hollander (1904) and Durme (1914) who insisted on the presence of psuedoreticulum in smaller oocytes at a particular age. Hollander, however, only studied chicks which were up to 20 days old and Durme was mostly concerned with sparrows and swallows.

Another point of interest was the orientation of the nucleus in the cytoplasm. In smaller oocytes (below 50μ) the nucleus was in the center of oocyte and comprised slightly more than half of the oocyte. Similar results were obtained by Hollander (1904) and Koltzoff (1938). When oocytes reached around 50 μ, the ratio of the cytoplasm to the

nucleus increased and the nucleus became eccentrically located. The location of the nucleus varied when the oocyte was between 200 μ - 2 mm, although generally it was located in the center. When the oocyte increased to 6 mm the nucleus again became eccentrically located and was either attached or partially pushed into the granulosa and vitelline membranes. Although Goldsmith (1928) indicated in oocytes of 37 day old chicks the nucleus was located in the center and eccentric at maturity, the results obtained in this study do not agree. Again it is not clear to which size oocytes he was referring.

Orientation of the nucleus in the oocyte cytoplasm has been explained in two ways: (i) According to Durme (1914) and Marza and Marza (1935), three step changes in the cytoplasmic activities follow three stages of growth. In the first and third stages, fats appear in larger amounts than protein in the oocytes. This fat in the cytoplasm makes it lighter than the nucleus which contains nucleoproteins. Thus the effect of gravity causes the nucleus to be deflected away from the center. (ii) Oocytes of approximately 40 - 50 μ diameter contain a Balbiani body impregnated with a mitochondrial cloud which appears pressing next to the nuclear wall (Brambell 1925, and Bellair 1965). Also in the last phase of growth there is an excessive appearance of white and yellow yolk in the oocytes. The pressure caused within the oocyte by the cytoplasmic inclusions at the point of its origin, pushes the nucleus in an opposite direction until it lies close to the outer wall of the oocyte.

A. Transitional Changes in Nuclei and Chromosomes.

Limited information is available on transitional changes in nuclei

and chromosomes during stages of vitellogenesis in fowl. On the basis of information obtained in this study, a general description of the transitional changes in nuclei and chromosomes, based on the growth phases (stages) of vitellogenesis proposed by Durme (1914) and Marza and Marza (1935), given in Table 6, will be described. The stages are: (1) Previtelline, (2) First stage of vitellogenesis, (3) Second stage of vitellogenesis, and (4) Third stage of vitellogenesis.

Table 6. Growth phases of vitellogenesis proposed by Durme (1914) and Marza and Marza (1935)

Growth phase	Diameter of oocyte (mm)	Cytology
First stage	0.05 - 0.2	Balbiani body, fat drops
	0.2 - 0.3	Dispersal of Balbiani body
	0.3 - 1.0	Dispersal of mitrochondrial body to periphery
Second stage	2.0 - 3.0	Vacuoles
	3.0 - 5.0	1st yolk appears
Third stage	6.0 - 9.0	Some white yolk
	10.0 - 35.0	White and yellow yolk, latebra, nucleus of pander.

1. Previtelline Stage.

Beyond 3 weeks of age oocytes of the previtelline stage, (20 - 40 μ in dia), were similar to the description given by Hollander (1904), Loyez (1906), and Durme (1914) in that some nuclei had an appearance of psuedoreticulum. The chromosomes appeared to be connected to the inner wall of the nucleus with finer filaments or chromatic trabeculae. Also the nuclear apparatus in the resting stage generally resolved single, eccentric, and nonspherical nucleolus (3 μ) plus a clump of chromatin masses scattered

throughout the nucleus (Figs. 34 and 38).

However, slightly larger oocytes of 30 - 50 μ had only diplotenic bivalents and their long axes had a segmented appearance. This segmented appearance was due to excessive condensation or uncoiling of chromomeres which were heavily stained.

2. First Stage of Vitellogenesis (50 μ - 2 mm).

It has been observed in this study that completion of Balbiani body formation synchronises with the initiation of lampbrush chromosome formation. Therefore, the first stage of vitellogenesis may be subdivided into: (1) Balbiani body formation (50 - 100 μ), and (2) Lampbrush formation (100 μ - 2 mm).

a. Balbiani Body Formation. During Balbiani body formation the nucleus is eccentric and its nucleoplasm is partitioned into ecto- and endonucleoplasm, with the chromosomal elements contained in the endonucleoplasm. As the oocyte matures the chromosomes orient, initially in a polar manner, and later parallel to the circumference of the nucleus.

When the oocytes reach 100 μ diameter, the nuclear wall becomes folded. Hollander (1904) and Durme (1914) reported that oocytes of this stage had double walls around the nucleus and they attributed the inner wall to the nucleus and the outer wall to the cytoplasm. As discussed in succeeding chapters this does not agree with the present findings obtained from using electron microscopy. Goldsmith (1928) reported the disappearance of the nuclear wall in the oogonium of 2 to 10 day old chicks. However, this appears to be incorrect as in the present study one week old oocytes contained a double nuclear membrane, which agrees with Greenfield (1966).

b. Lampbrush Chromosome Formation . The earliest age at which nuclei containing the lampbrush chromosomes was observed was eighteen days after hatching in which the oocyte was 148 μ, nucleus was 49 μ and nucleolus was 7 μ.

Similar results were obtained by Hollander (1904) who observed lampbrush chromosomes with a large nucleus in 20 day old chicks. Sonnenbrodt (1908) also observed in 90 - 140 μ oocytes of 21 day old chicks, barb chromosomes and a single nucleolus which later fragmented. Durme (1914) and Brambell (1925) also reported lampbrush chromosomes and a single nucleolus in 70 μ oocytes (35 μ nucleus) of 21 day old chicks.

At an advanced age (25 days) the smallest oocytes (110 μ , nucleus 48 μ) resolved lampbrush chromosomes. Regardless of the age of the fowl many oocytes of 150 - 250 μ showed clear lampbrush chromosomes and this was the size when the Balbiani body disposed. Koltzoff (1938) sketched classical diplotene chromosomes with neat bivalents in 80 - 150 μ oocytes of laying hens and reported the appearance of lampbrush chromosomes in his second period of chromosomal transformation (oocytes of 150 - 500 μ diameter).

Segmentation of chromosomes leading to separation and disintegration of chromomeres by prior breaks at inter chromomeric points (Loyez 1906, Sonnenbrodt 1908, and Koltzoff 1938) does not seem valid on the basis of the present findings. The segmented appearance depends on the plane the lampbrush or pre-lampbrush chromosome axis was cut in sections.

Even though Durme (1914) found multiple nucleoli in the sparrow and the swallow, in this study they were not very obvious in the early stage of the first period of vitellogenesis. However, as the oocyte enlarges beyond 150 μ, the nucleoplasm tends to be coarser and granular.

By the time the oocyte reaches about 1 mm the entire nucleoplasm is covered with larger granules or multiple nucleoli which later increase in dimension and density (number) as maturity advances.

As the oocyte increases in size to a maximum of about 200 µ the ectonucleoplasm boundary increases to its maximum. The boundary of demarcation established between ecto- and endonucleoplasm is determined by the long axes of the chromosomes which lie parallel to the circumference, consequently leaving a gap between the endonucleoplasm and the nuclear membrane. This observation is in agreement with Sonnenbrodt (1908) and Durme (1914).

3. Second Stage of Vitellogenesis.

The second stage of vitellogenesis is characterized by vacuole formation, appearance of yolk in the cytoplasm and a large number of granules (nucleoli) in the nucleus. In the present study this stage was observed in 4 week old oocytes of 456 µ diameter although according to Marza and Marza (1935) this stage shbuld have been observed in oocytes larger than 1 mm diameter. At the 2nd stage of vitellogenesis most of the oocytes contained lampbrush chromosomes and some appeared as fragmented pieces of chromatic axes. However, the number of lampbrush chromosomes per nucleus was reduced to 1 - 4 which contained defined lateral loops studded with granules. In their staining behaviour, and in size the nucleoli resembled the granulations located on the lateral loops of the lampbrush chromosomes.

4. Third Stage of Vitellogenesis.

In the third phase of vitellogenesis the entire nucleoplasm consisted of vacuolar reticulum, presumably of chromatic fibers. The

chromatic filaments attached to the nucleoli decreased in size during the development of oocytes. Isolated fragments of chromomeric origin were observed embedded in nucleoplasm. Clumps of chromatic segments were localized in the nucleoplasmic reticulum thereby forming irregular shapes of chromomeres. At later stages of development, until the end of maturation, the nuclei were filled with nucleoli and granules of various sizes.

B. Sequence of Transformation of Lampbrush Chromosomes*.

In growing, as well as in mature fowls, oocytes above 50 μ contain diplotene chromosomes whose axes have started orientating in different directions. At the same time as the chromosomes are being oriented the nucleoplasm differentiates into ecto- and endonucleoplasm and the nuclear wall becomes folded and corrugated. The chromosomes which are moving towards the periphery of the ectonucleoplasm have characteristics which usually occur prior to lampbrush chromosome formation. In oocytes above 100 μ the main chromosomal axes consists of long fibers with chromomeres which stain heavier and appear larger than the width of the axes.

When oocytes are approximately 150 - 200 μ or 18 days and older, lampbrush chromosomes are resolved in the nucleus. In most sections, they appear to be synchronous in development (Figs. 33, 37, and 51) in that at least 12 lampbrush chromosomes could be observed throughout the nuclear field. But in oocytes above 1 mm dia only a few chromosomes were observed in the lampbrush stage.

Although it is impossible to follow the same chromosome during the

---

*Fig. numbers are given in this section to aid in understanding.

complete process of transformation and record a stepwise change in its structural details, one may consider a classical diplotenic bivalent (Fig. 28) as a starting point. The long axis becomes uniformly granular. Later, the chomomeric regions become uncoiled or diffused and appear at several places as thick bumps (Fig. 31). That is, the entire longitudinal axis of the chromosome transforms intermittently into coarser and thinner segments (Fig. 32). At this stage a centrosphere-like spot appears in the cytoplasm (Fig. 36) which may or may not be related with the events of the barb formation.

The chromomeres grow, unfold, and expand transversely along the long axis of the chromosome (Fig. 50). They extend very fine lateral processes which in the beginning appear fuzzy and diffused (Figs. 36 and 51). Simultaneously,some chromomeres project transversely, coarser and stumpy extensions of side arms (Figs. 47 and 52). At this stage an analogy may be made to the cervical region of the vertebral column.

The axial filament of the chromosome appears to be discontinuous (Figs. 47 and 52). This appearance of discontinuity is caused by excessive bulging (uncoiling) of chromomeres which when sectioned on horizontal plane of the chromosome appear as discrete chromomeres or a sectioned chromosome discontinued at the inter chromomeric regions.

Next, the lateral loops further elongate into a very fine process, occasionally there are two such processes on each side of the chromomeres (Figs. 45 and 53). These side loops contain fine granules arranged singly in a linear order along their axes (Fig. 53). Such granules originate along with the lateral extension of the chromomeres (Figs. 56 and 58) and later spread with the entire extensions of the lateral loops.

Later these loops become wider along their axes, their granulation appears coarser (Fig. 57) and eventually they become shorter (Fig. 56) in appearance. The chromomeres along the axes of the chromosomes still stain very heavily but show signs of splitting longitudinally (Fig. 43).

At a more advanced stage, broken pieces of chromatic segments with chromomeric points on their axes are visible (Fig. 59). It is difficult to say whether these side loops of variable sizes and shapes formed from the linear array of chromomeres, are still attached to the chromosome on its long axis.

The resemblance in appearance of the granules of lateral loops to those that were dispersed in the nucleoplasm is suggestive that the loops become ultrafine fibers which spread throughout the nucleoplasm. In the fixed stained sections of larger oocytes these loops are difficult to spot. Their presence is further masked by the accumulation of nucleoplasmic granules (peripheral nucleoli) which were formed and later either dispersed or remained attached to the lateral loops of chromosomes.

C. Changes in Oocyte Parameters.

Another question is whether there is a relationship between age and size of oocyte and also between growing oocytes and nuclei. Reports from the literature are presented in Table 7.

In the present study, 10 - 20 μ oocytes containing 7 - 11 μ nuclei were observed as early as 7 days. An oocyte larger than 30 μ was the smallest observed in the population of oocytes which existed from 4 weeks until maturity. Although the fixed and stained oocytes were expected to demonstrate shrinkage in the walls, and the nucleus, etc., this was not observed in comparing 1 mm oocytes of 16 weeks

Table 7. Relationship of age to oocyte and nuclear diameter

Author	Year	Age (days)	Oocyte (μ)	Nucleus (μ)
Brambell	1925	4	10 - 22	6
		21	37 - 40	20 - 35
		42	38 - 380	2/3 of oocyte
		77	50 up	
Sonnenbrodt	1908	14	60 - 80	
		21	90 - 140	
		180	750 - 960	
Olsen	1942	Young chick	90	36
		Mature	1.7 mm EF	250

and older birds to laying hen oocytes of the same sizes. The size relation of nuclear size to egg follicle of the hen agreed closely with the value reported by Sonnenbrodt (1908) and Olsen (1942).

Depth of culumnar epithelium increased in oocytes growing from 50 to 200 μ diameter, and reached a maximum of 18 - 19 μ. Similar results were reported by Durme (1914), Marza and Marza (1935) and Bellair (1965).

The size relationship of oocytes with the corresponding ages of growing chicks does not agree with Brambell (1925), whereas the relationship between oocyte and nuclear sizes as indicated by Brambell (1925), Sonnenbrodt (1908), and Olsen (1942) agree closely with the present findings.

II. Isolated Nuclei.

A. Morphological Considerations.

Dodson (1948) reviewed contemporary authors and concluded that chromosomes persist during meiosis (oogenesis) in amphibians and fish, more precisely at and after the lampbrush stage. In chickens, Loyez (1906) and Sonnenbrodt (1908) were not certain about the existence and morphological continuity of meiotic chromosomes after lampbrush formation. They maintained that chromosomes reappear at the time of maturation divisdon, that is, at the first meiotic metaphase. Some early cytogeneticists claimed disappearance of nucleoprotein in this phase (Koltzoff 1938), and even Brachet (1929) in his early reports could not detect Feulgen positive reaction in the lampbrush stage of chromosomes. Later, after finding nucleoprotein present, Brachet (1940) reversed his opinion and concluded that the main axes of chromosomes remained Feulgen positive at all times even though the side loops of lampbrush chromosomes may not.

The results of this study tend to confirm the conclusions of Dodson (1948) and Brachet (1940) for the amphibians and Durme (1914) for the fowl that the chromosomal elements remain visible during the entire maturation cycle of oogenesis (meiosis). In previous parts of this study, sections of lampbrush chromosomes were demonstrated in large egg follicles from 18 days until maturity. These lampbrush chromosomes have also been manually isolated from hen egg follicles of 1 - 3 mm dia. If a section of isolated lampbrush chromosomes (Figs. 67, 70 and 73) was cut axially, the resulting structure would have an appearance identical to the lampbrush shape shown in the earlier stained sections (Figs. 44, 45, 53

and 57).

Results of section studies viewed through an electron microscope disagree with the findings of Greenfield (1966) only on the point that the fine structure of meiotic chromosomes in oocytes from chickens older than 3 weeks is visible but the oocyte has to be very small, i.e. below 100 Å dia.

Although Greenfield (1966), who was using electron microscope, did not observe chromosome filaments in large oocytes (80 - 100 U) after 3 weeks of age, in this study using light and phase microscope, lampbrush chromosomes were observed in large oocytes obtained from 18 days and older chicks.

Other fibrillar structures isolated from advanced oocytes (above 1 mm egg follicle) which are described in this study as lumpy loops, mulberry type elements, and nucleolar bearing lampbrush chromosomes, may be regarded as modified forms of lampbrush chromosomes or their lateral loops because their axes were studded with chromomeric-type beads, had Brownian movement in the living state, and were dissociated by DNase treatment. Not only did the mulberry type elements demonstrate Feulgen positive reaction but also other fibrillar elements demonstrated labelling of tritiated Actinomycin D. The whole mount preparations of the chromosomal elements had a basic strand of 250 Å. These findings are in agreement with those of Baker and Franchi (1967), Wolfe and Bernard (1965), Wolfe and Hewitt (1966), Ris and Chandler (1963), Ris (1966), Gall (1966b) and Bol et al. (1969) who reported similar dimensions of the fine structure of the meiotic chromsome in different living systems. Presence of chromosomal elements, including the lampbrush chromosome, is evident during meiotic prophase of the chicken

oocyte and to the end of the reproductive life span of the hen.

The breakdown of lampbrush chromosomes into various elements as described and the simultaneous appearance of peripheral nucleoli have raised a question as to whether there is a need of defining a new stage (phase) of chromosomal movement occuring after diplotenic lampbrush and diakinesis, particularly in animals which have a long generation interval and yolky oocytes. Diplotene and lampbrush chromosomes in a classical configuration could still be resolved in the egg follicles up to 3 mm dia, and lampbrush and dictyate chromosomes can be recognized in fowl oocytes up to 6 mm egg follicles. In this range of oocytes the nucleolar lampbrush chromosomes appeared as separated sister chromatids. Therefore, in advanced oocytes, above 3 mm egg follicles, the conventional quarternary structure of the chromosomes changes asynchronously into heteromorphic fibers (elements) of finer depth and thickness which constitute the pseudoreticulum of the nucleus. This agrees with Bal et al. (1969), who studied the nuclear changes during meiotic prophases of Arbacia puntulata primary oocytes. In growing oocytes, above 500 μ dia, the lampbrush and fine amorphic fibers of chromosomes are associated with production of multiple micronucleolic which become more obvious in larger oocytes when the giant nucleolus no longer exists. Thus the post lampbrush stage of advanced oocytes may be analogous to somatic interphase chromosomes with the exception that the chromatin, in this case, does not replicate DNA but is involved with production of multiple nucleoli, in other words, synthesizes RNA. Prior to first maturation division (reduction division) these irregular fibers (250 Å) of chromosomes may end up in jumbled masses of chromatin which constitute the basic material for the reduction division (Durme 1914

and Olsen 1942).

B. Strandedness of Chromosomes.

The lampbrush chromosome is regarded as a diplotenic bivalent in which each of the bivalents bear lateral loops and granules. Each monovalent consists of two sister chromatids of which it is considered that the non-sister chromatids are involved in chiasmata formation. The chromosomal structures are quite complex and many descriptions of the coiling pattern and the strandedness of mitotic chromosomes have been made, e.g. Swanson (1957), Kauffman (1948), Darlington (1955), Bajer (1965) and Cole (1967).

On several occasions in this study it was observed that the coiling of the chromatids was plectonemic; and under phase at the terminal ends of the bivalents two chromatids could be observed in each homologue of the bivalents. The observations therefore agree with Bajer and Allen (1966) and Gimenez-Martin and Lopez-Sacz (1965), suggesting that the coiling of the chromatid strands was plectonemic (co-helical or relationally twisted pair as in DNA double helical molecule) rather than paranemic (independent).

An alternative interpretation of chromosomal coiling was given by Colombo (1955), Cole (1962), and Inoue and Sato (1963). They conceived of a single basic fiber of chromosome which is packed in the form of coiled coils, supercoils, or by various kinds of folding such as a quasi-random coil indicated in DuPraw's (1965) folded fiber model. Although it is still controversial (Lafontain and Ris 1958 and Ris 1966), most evidence indicates that there is only one DNA double helix per chromatid in the lampbrush chromosome (Peacocke and Drysdale 1965

and Miller 1964a, 1965a).

Callan and MacGregor (1958) and MacGregor and Callan (1962) treated the isolated lampbrush chromosomes of newts with DNase. The DNase severed either the axis of the loop or the main axis of the chromosomes. This agrees with the findings of the present study. From the measurement of reaction kinetics in which the loops were broken by DNase, Gall (1962a, 1963) calculated that the axis of the loop is one double helix of DNA, while that of the main axis of the lampbrush chromosome is a pair of DNA double helices. On removing RNA and protein from the loops a fine fibril of 40 - 50 Å dia, presumably composed of a DNA double helix, was recorded by Miller (1962a). By electron microscopy followed by enzyme treatment to lampbrush chromosomes, Miller (1965a) demonstrated the minimum diameter of the DNase sensitive lateral loop axis (one chromatid wide) was 20 - 30 Å , whereas DNA sensitive interchromomeric strands in the main lampbrush chromosomal axis (two chromatids wide) measured about 30 - 50 Å. However, the finest fibers of the lampbrush chromosome as commonly observed in this study measured about 200 - 250 Å wide. Therefore, critical information is not available to clarify whether this strand of 200 - 250 Å is a single or multiple DNA double helix containing fiber. Nevertheless, the basic nucleoprotein fibers shown in the whole mount electron micrographs appear similar in structure and measurement to those reported by Gall (1966b).

C. Linear Organization of Chromosomes.

In the present study, light micrographs of the lampbrush chromosomes clearly demonstrated transverse bridges along the main axis.

From whole mount preparations the dictyate or late lampbrush chromosomal elements were resolved under both phase and electron microscopy to be separated from each other. Because isolated mulberry and other types of chromosomal elements per nucleus are much more than would be accounted for by 80 chromosomes (2n = 80 chromosomes in fowl), they are clear evidence of separation (not detachment) of chromomeres from a main axis whose lateral loops and folds have branched into various directions. It may be argued that the limit of resolution in not only the light microscope but also the electron microscope was not sufficient to detect the presence of the extended interchromomeric regions which presumably remained a pair of DNA double helix as shown in amphibians by Miller (1965a).

Furthermore, in the present study, DNase only was ineffective in a freshly isolated lampbrush chromosome unless a pretreatment of a proteolytic enzyme was applied to it. The synergistic effect of both enzymes when applied one after another severed transversely the long axis of the chromosome at several places. It may be that the regions of the axis broken by the two enzymes had less heavy deposits or blockage by histones and other proteins than those segments of the chromosome which remained intact. Coleman and Moses (1964) studying the fine structure of the synaptic chromosome in the domestic rooster observed a similar situation. They considered materials other than DNA were present in the microfibrils and showed that little alteration in appearance of chromosomes resulted from only DNA removal.

The present findings of enzyme action are in agreement with Callan and MacGregor (1958), MacGregor and Callan (1962), and Gall (1963). The action may be slightly different in the fowl than in newt

because the long axis was not severed by DNase only (Coleman and Moses 1964).

The evidence supports the idea that the chromonema is continuous and extended throughout the entire body of the chromosome. It appears that the point where the DNase breaks the main axis which is composed of microfibrils containing DNA strands folded in the trypsin or pronase sensitive matrix was of a minimum thickness so as to be acted upon by both the pronase and DNase. These points in chromosome axis must have been the interchromomeric regions of the monovalents and most likely represented a short length of DuPraw's 'folded fiber' chromosome model. This analogy is further supported by the estimate of the basic strand of lampbrush chromosome, obtained by sections and whole mount electron microscopy, of about 200 - 250 Å. This estimate is very close to estimates reported by DuPraw (1965, 1966) for his folded fibers and is also in agreement for the giant chromosomes of Diptera (Yasuzumi et al. 1951); for the lampbrush chromosomes of Triturus, (Tomlin and Callan, 1951); for Triturus, Nicturus, Amphiuma1, and Lilium (Ris 1955, Lafontaine and Ris 1958, Ris and Chandler 1963, Ris 1966) and meiotic chromosomes of other species (Wolfe and Bernard 1965, Gall 1966b, Wolfe and Hewitt 1966, Wolfe and Martin 1968, Baker and Franchi 1967, and Bal et al. 1969). The micrographs of sea urchin oocyte chromatin by Wagenaar and Mazia (1969) also contained similar chromosomal microfibrils.

The morphology of nucleoli bearing lampbrush chromosomal elements appears to be mostly in the late dictyate stage. It appears that the lateral projecting loops from the chromomeres are stretched

and pulled away from the main axis of chromosome. Using electron micrographs of human primodial oocyte sections, a similar pattern of the early prophase chromosomes has been proposed by Baker and Franchi (1967).

Isolated nuclei from maturing oocytes (about 3 mm), when the fowl oocyte approaches second to third stage of vitellogenesis, are mostly in post diakinesis. The micrographs (phase and electron whole mount) resemble very closely the nucleolar chromosomes of Wilson and Morrison (1966). However, the scattered lateral loops or branches of lampbrush chromosomes, bearing chromatic nucleoli are extensions of chromomeres, which are not resting, but probably are involved in intensive synthesis of nucleoli (RNA) even in diakinesis.

In advanced oocytes, the observations of nuclei containing thousands of spheroidal nucleoli and the nucleolar bearing chromosomal strands are quite similar to what Wolfe (1967) resolved for chromatin spheres and strands from isolated macronuclei of Paramaecium aurelia.

D. General Morphology of Lampbrush Chromosomes.

Many investigations on lampbrush chromosomes concerning the nature of the chromomeres, lateral loop formations associated with chromomeres, chromonema, and the chiasma formation have been made. However, it is now established that the chromomeres, the lateral loops, and the chiasma are continuations of the chromonema (basic chromosomal microfibril) which have been transformed into various shapes by folding and unfolding (Lafontaine and Ris 1958; Wishnitzer 1961; Srivastav and Bhatnagar 1962; Callan 1963, 1966; Gall 1966a; and Ris 1966).

However, one question which has not been clearly answered is

whether the structure of one arm of a lampbrush bivalent consists of double chromatids (sister-chromatids) or multistrands. Although double chromatids are commonly assumed, this has not been clearly demonstrated in a single photograph in the literature except camera lucida drawings. A confusing point concerning this is the finding of Callan (1959) that the interchromomeric regions in the newt lampbrush chromosomes are single stranded, although Srivastava and Bhatonagar (1962) reported observing two discrete chromomeres lying in juxtaposition on a monovalent chromosome. However, they proposed that each chromomere was associated with a separate chromatid and that they had occasionally noticed double chromatids in the same monovalent of lampbrush chomosomes.

The micrographs of the lampbrush chromosomes in this study of the stained sections, light microscope; the isolated chromosomes, phase microscope; and the whole mount preparation electron microscope; have clearly demonstrated that each monovalent consisted of two longitudinal strands (two chromatids). Although hard to resolve, the doubleness along the entire axis, the presence of two chromomeres side by side supports the presence of two chromatids in each homologue of the bivalents because if there are two chromomeres side by side the inter chromomeric strand cannot be single.

A clue to the mechanism of chiasma formation may be obtained from study of the meiotic chromosomes. Although the crossing of one homologue with another homologue is commonly observed in the isolated meiotic bivalents, lampbrush chromosomes have not yet yielded decisive evidence regarding the detailed structure of chiasmata. Gall (1954a)

and Wischnitzer (1957, 1961) have shown that the two homologues of lampbrush chromosomes are often connected with chiasmata through a common chromomere and occasionally even through a centromere. It is not possible, on the basis of the photographs and the phase observations obtained in this study, to determine whether the two homologues crossed at the centromeres or a chromomere. However, information on pigeon metaphase chromosomes by Nebel and Coulon (1962) suggests that the kinetochores at chiasmata are set deep or sunk into the body core of the chromosome, which supports the view of Gall (1954a) and Wischnitzer (1957, 1961).

E. Working Model of Lampbrush Chromosomes in Fowl Oocyte.

At present there are three major interpretations of the structure of lampbrush chromosomes:

1. Duryee (1941, 1950) indicated each chromosome is composed of at least one and at most two fine chromonemata along which are located Feulgin positive chromomeres. He considered the lateral loops were out-growths of the chromomeres which were periodically shed and replaced by a new out-growth (projection) on the chromosomes. This was supported by Guyenot and Danon (1953), Gall (1954a), and Wischnitzer (1957).

2. Ris (1945, 1955) postulated that the lateral loops were parts of continuous chromonemata rather than a secretion product of the chromomere. He believed that the chromonema is composed of bundles of microfibrils chromonemata (multistranded). Results of electron microscope studies tend to support this (Lafontaine and Ris 1958).

3. Callan (1955) and Gall (1956) reviewed previous experiments and proposed a model which was a compromise between the models proposed

by Duryee (1941, 1950) and Ris (1954, 1955). In their model the homologues are composed of two expanded chromatids a few hundred angstrom thick, which bear chromomeres in a continuous row along the entire length of chromomatid and the side loops by perhaps a coiling and uncoiling arrangement. Wischnitzer (1961) has supported this model on the basis of electron microscopic, micromanipulation, and cytochemical evidence present in the literature. At the present time this model is widely accepted and is known as the double chromatid-paired loop hypothesis of the lampbrush chromosomes.

The findings of the present investigation agree, in general, with the model proposed by Callan (1955) and Gall (1956) and the proposal of Lafontaine and Ris(1958) that loops are developed by extension or uncoiling of the chromonema at the point of chromomeres. However, it is not clear if each chromomere produces only single or double loops. The observations of a chromomere (section material) indicated several rossette forms of loops. In isolated chromosomes, although the places of origin of the loops are hazy, in one of the bivalents the loops could be counted as four per chromomere. It is quite possible that the loops are formed around the axis of the chromomere and there should be a minimum of two loops per chromatid. Whether this finding would invalidate the concept of one chromonema containing one DNA double helix molecule or several DNA in each chromonema is not clear at this moment.

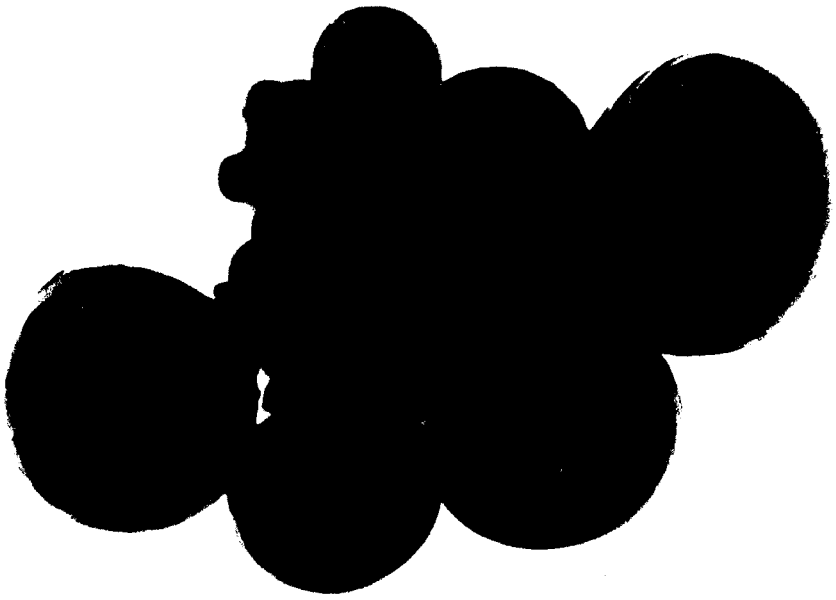
Furthermore, in the lampbrush chromosomes of the fowl, it appears that one loop may be extended and pulled out so much from the chromomere that it may appear to lose the continuity of chromonema. In this study lateral loops of the lampbrush chromosomes as

demonstrated through light microscopy and phase microscopy probably have a different morphological organization. In fowl the entire axes of the lateral loops were studded with chromatic granules in a single file. This single file may be a simple aggregation of nucleolar RNA materials. If such is the case then the lateral loops of fowl would differ from amphibians and fishes in having several loci of RNA synthesis in each lateral loop.

An additional modification in the lampbrush chromosome structure of fowl appears in advanced stages of the oocyte when neither lampbrush chromosome nor a classical diakenetic configuration of chromosomes are identifiable in the nucleus. This stage occurs before the clumping of chromosomes prior to first reduction division. It appears as if the separation of each homologue of the bivalent is also followed by separation (unzipping) of the sister chromatids from which the majority of chromomeres unfold and extend long lateral loops. It is at this stage when the nucleus is full of long ribbon like fibres of equal width spread in all possible directions and on whose axes there are chromomeres and chromatic nucleoli or micronucleoli. This stage may be regarded as analogous to interphase stage of somatic cells where chromatin is not involved in replication of DNA but synthesis of nucleolar RNA and which later ends as a stage where clumps of chromatin masses are observed in the nucleus before the first meiotic mitosis ensues (Olsen 1942).

Fig. 11. Incised ovary of laying hen showing pedunculate large egg follicles and blood supply. Magnification Close up.

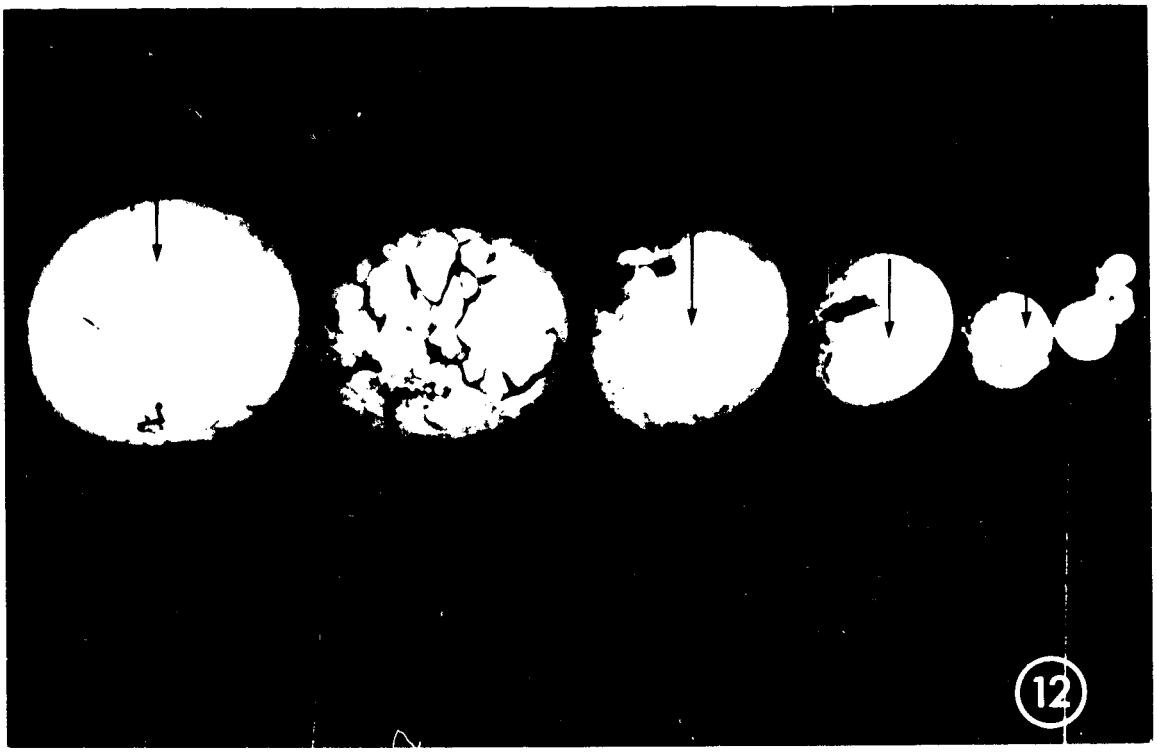
Fig. 12. Egg follicles isolated from a laying hen ovary. Arrows indicate nucleus position. Magnification Close up.



11

12

(11)



(12)

Fig. 13. Ovaries dissected from 4 week old (A), 8 week old (B), and 12 week old (C) female chicks. Magnification Close up.

Fig. 14. A part of ovary dissected from 16 week old chicken. Magnification Close up.

Fig. 15. A part of ovary of 16 week chicken. Note the egg follicles are pertinent over the ovarian ridges. The arrow points to a ridge. Magnification approximately 20 X.

Fig. 16. Plucked egg follicles from 20 week chick ovary. Note the oocytes contained within each follicle are clearly delimited from the surrounding follicular walls. Follicle labelled X emptied after isolation of the oocyte. Magnification approximately 40 X.

A
B
C
13
14
15

Fig. 17. An incized rectangular piece of egg follicle with intact layers laid inside down. Arrow points to position of the nucleus, yellow yolk (Y). Magnification approximately 30 X.

Fig. 18. Rectangular piece laid inside up. Note the position of nucleus in the centre. Nucleus covered by yellow of yolk and the intact follicular membranes below. Magnification approximately 70 X.

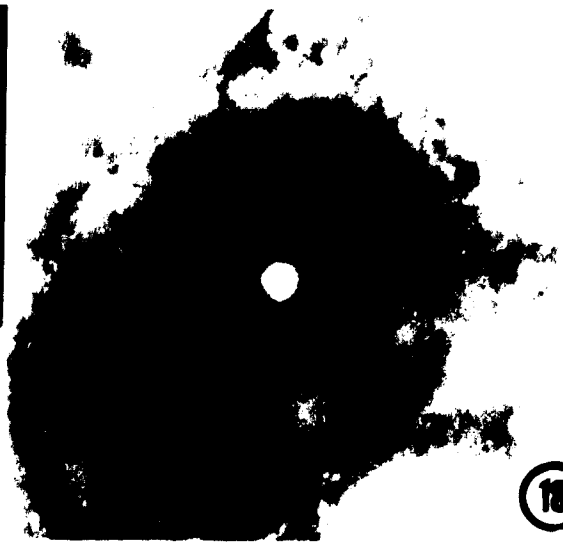
Fig. 19. Rectangular piece from which peritonium, theca externa, interna removed from beneath the nucleus, and yellow of yolk from the top of nucleus. Note nucleus surrounded by bulged ring of white yolk (Wh). Magnification approximately 70 X.

Fig. 20. Freshly isolated nucleus (N) which was half covered by white of yolk (Wh) inside the oocyte and half embedded in the exvaginated layers of follicular epithelium. Magnification approximately 200 X.

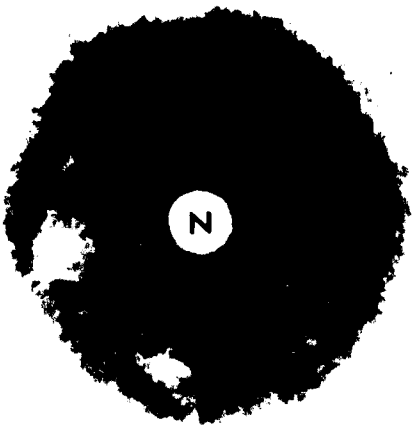
Fig. 21. A clean isolated nucleus - 330 u. Magnification approximately 200 X.

Figs. 22, 23. Clean nuclei. Note how the nuclear wall was torn apart. Magnification approximately 200 X.

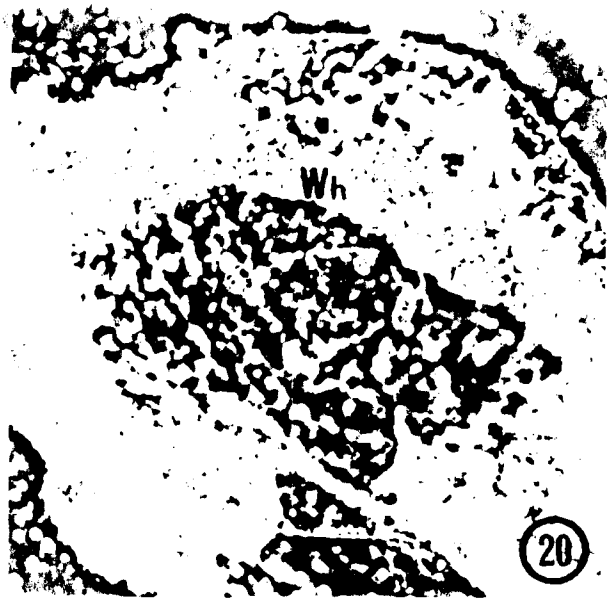
17



18



19



20

21

22

23

Fig. 24. Actively proliferating sub-epithelial cells. 8 μ section of 7 day ovarian tissue. Magnification approximately 600 X.

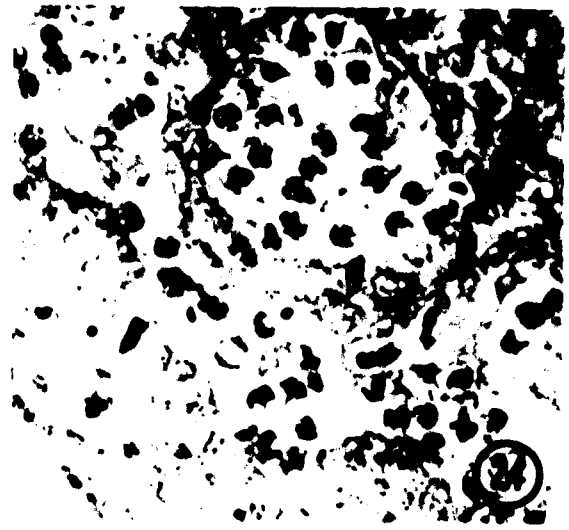
Fig. 25. Extra follicular oocyte. Nucleus 7.5 μ, nucleolus 3 μ. Arrow indicates follicular layer in the process of encapsulation. Section of 7 day ovary. Magnification approximately 1,730 X.

Fig. 26. Small 30 μ oocyte, nucleus 18 μ; showing chromosomes attached to the nuclear wall. 8 μ section of 7 day ovary. Magnification approximately 1,730 X.

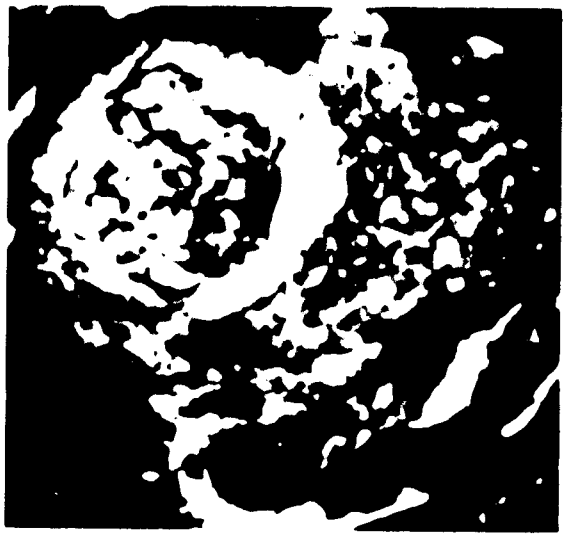
Fig. 27. Two oocytes surrounded by a common follicular layer capsule (arrow); showing nucleolus 2.5 μ and condensed chromatin along the chromosomal axis. 8 μ section of 7 day ovary. Magnification approximately 1,730 X.

Fig. 28. Large 40 μ oocyte, nucleus 20 μ; showing diplotene chromosomes with condensed chromatin and a 3 μ nucleolus (NL). 5 μ section of 7 day ovary. Magnification approximately 1,730 X.

Fig. 29. Section of ovary from 25 day chick showing larger oocytes migrating towards medula of the ovary. 8 μ section. Magnification approximately 200 X.


24


25




27


N
NL
28

Fig. 30. Oocyte; 29 μ, nucleus 20 μ; forming a ball of chromatin network in A. Diplotene chromosomes with chromomeric condensations along the axis in B; one of the chromosomes attached to 4 μ nucleolus. Note separation of nuclear wall from cytoplasm. 8 μ section of 15 day ovary. Magnification approximately 1,700 X.

Fig. 31. Oocyte; 65 μ, nucleus 25 μ and nucleolus 2.5 μ. Arrow indicates a nucleolar like condensation at the crossing of two bivalents. 14 days ovary. Magnification approximately 1,700 X.

Fig. 32. Oocyte; 50 μ, nucleus 25 μ; showing ecto-(EC) and endo-EN) nucleoplasmic zones. Note heavy condensation along the chromosome axis. 8 μ section of 18 day ovary. Magnification approximately 2,000 X.

Fig. 33. Oocyte; 148 μ, with 50 μ nucleus (N) and a large 7 μ nucleolus (NL). Arrow indicates one of the lampbrush chromosomes radially arranged in the endonucleoplasm. 8 μ section of 18 day ovary. Magnification approximately 1,900 X.

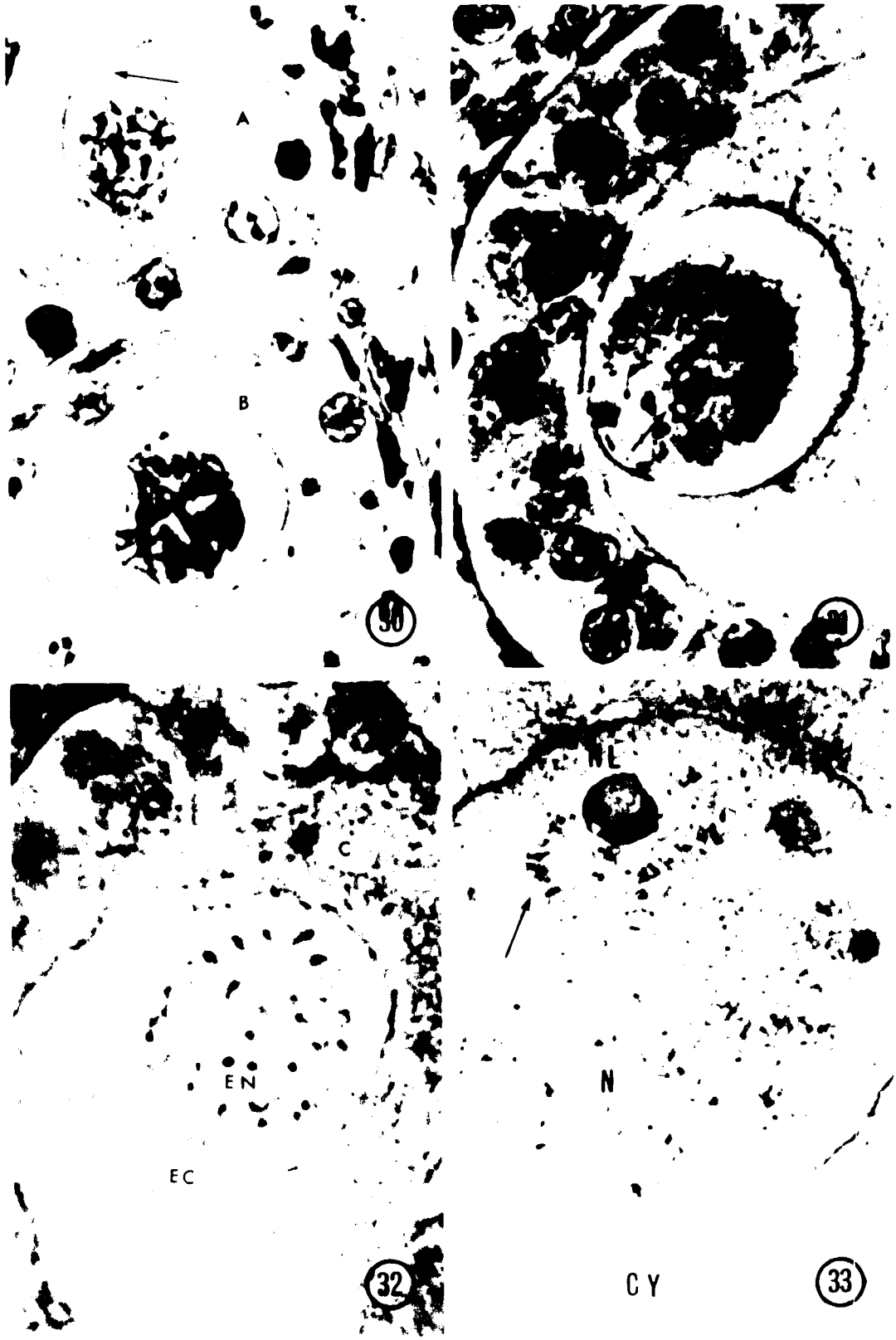

A
B
30
31
C
EN
EC
32
NL
N
CY
33

Fig. 34. A typical resting oocyte; 28 μ, nucleus 15 μ; containing 3 μ nucleolus. 8 μ section of 25 day ovary. Magnification approximately 1,530 X.

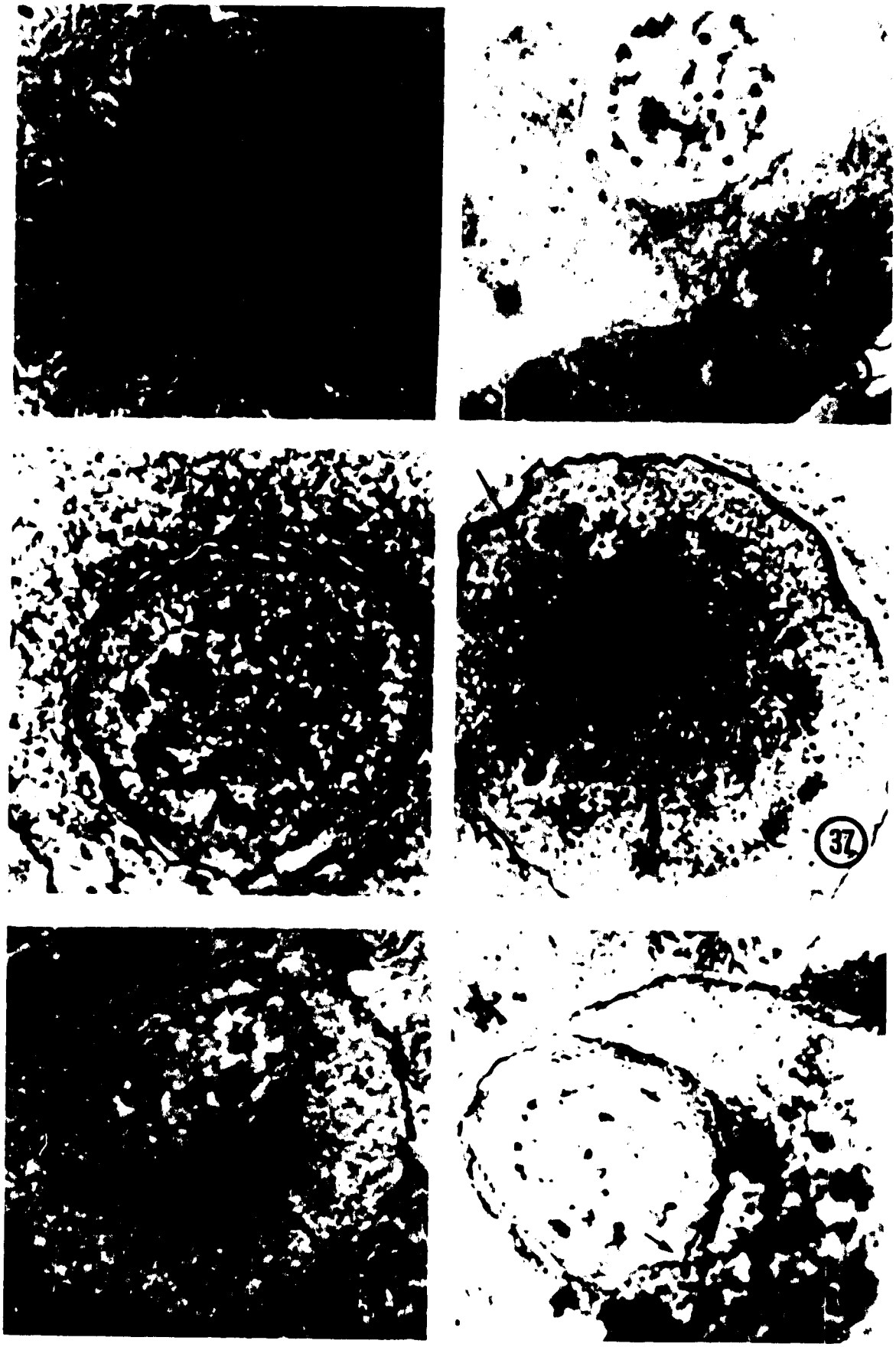
Fig. 35. Oocyte; 49 μ, nucleus 23 μ. 8 μ section of 25 day ovary. Magnification approximately 1,530 X.

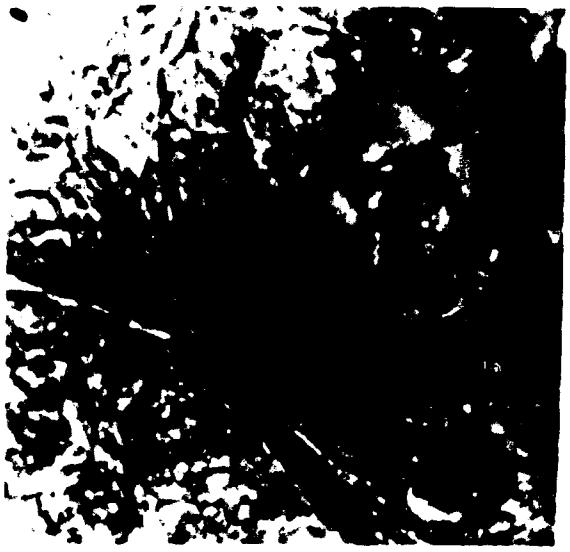
Fig. 36. Small 67 μ oocyte from ovary of 25 day old chick containing 30 μ nucleus. Arrow indicates diffused boundaries of the chromomeres. 8 μ section. Magnification approximately 1,530 X.

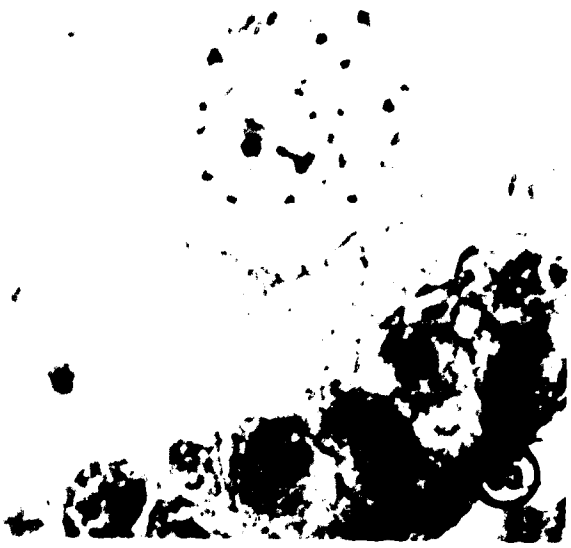
Fig. 37. Oocyte; 110 μ, nucleus 48 μ; containing lampbrush chromosomes in a polar and horizontal arrangement. Arrow indicates section across chromomere of polar chromosome. 8 μ section of 25 day ovary. Magnification approximately 1,530 X.

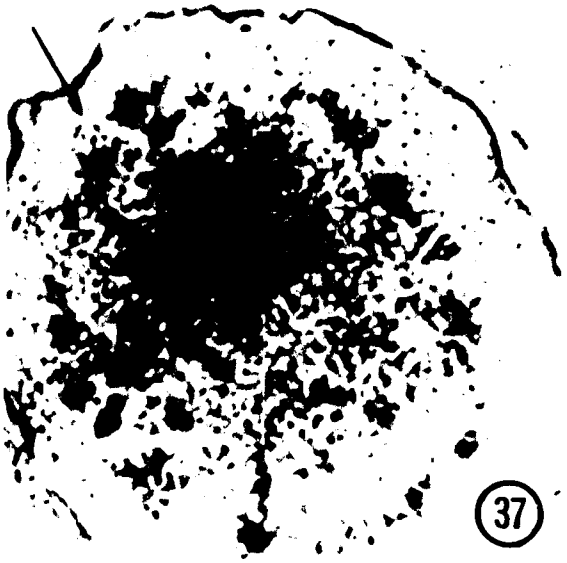
Fig. 38. Small oocyte; 38 μ, nucleus 22 μ; showing a large nucleolus. Resting oocyte with Balbiani body (B) in the cytoplasm. 8 μ section of 4 week ovary. Magnification approximately 1,530 X.

Fig. 39. Oocyte; 52 μ, nucleus 27 μ; showing chromosome in the diplotene configuration. Arrow indicates association of nuclear wall with Balbiani body. 8 μ section of 4 week ovary. Magnification approximately 1,530 X.


37






37

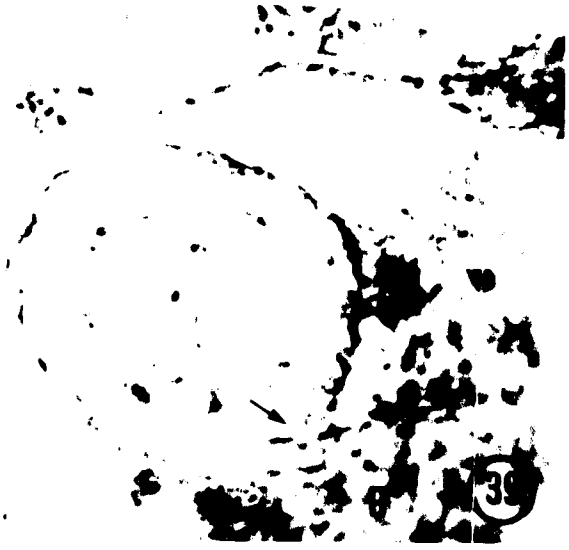


Fig. 40. Small 47 μ oocyte, nucleus 26 μ. Arrow indicates deeply stained follicular epithelium. 8 μ section of 7 week ovary. Magnification approximately 1,730 X.

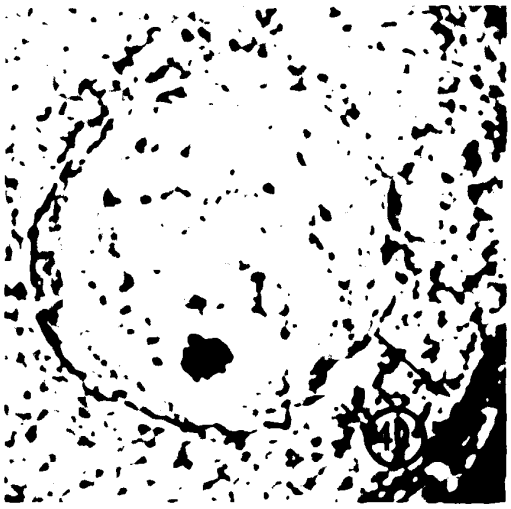
Fig. 41. Active 51 μ oocyte; nucleus 21 μ (N), showing diplotenic chromosomes. Note Balbiani body (B) of cytoplasm (CY) associated with the nucleus. 6 μ section of 7 week ovary. Magnification approximately 2,300 X.

Fig. 42. Large 92 μ oocyte, nucleus 36 μ. 8 μ section of 7 week ovary. Magnification approximately 2,300 X.

Fig. 43. Oocyte; 400 μ, nucleus 88 μ. Arrows indicate a lampbrush chromosome axis which is discontinuous in the inter-chromomeric regions and split chromomeres. Compare nucleoplasmic granules inside A to Fig. 45. 8 μ section of 12 week ovary. Magnification approximately 2,040 X; Fig. A, magnification approximately 1,530 X.

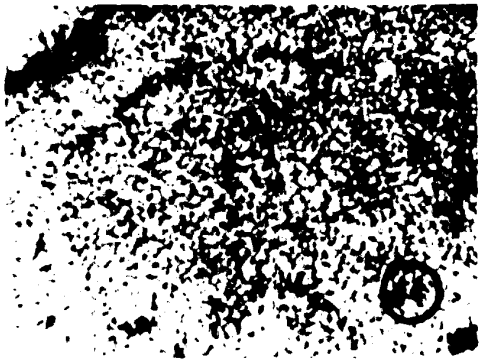
Fig. 44. Large 520 μ oocyte, nucleus 102 μ. Arrow indicates chromosome in lampbrush stage. 8 μ section of 7 week ovary. Magnification approximately 1,530 X.

Fig. 45. Oocyte; 400 μ, nucleus 90 μ. Arrow indicates lampbrush chromosomes throwing loops from chromomeres and chain of chromomeres appear disconnected along chromosomes' axes. 8 μ section of 12 week ovary. Magnification approximately 1,530 X.




CY
N


A



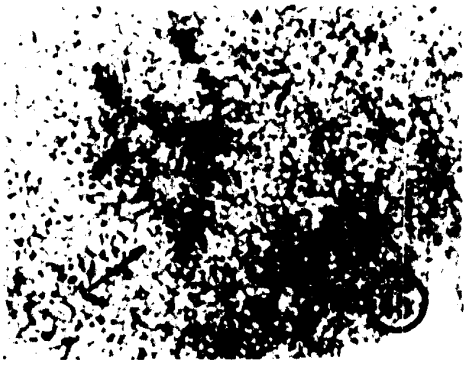


Fig. 46. Oocyte; 350 μ, nucleus 89 μ. Arrow indicates lateral loops originated from chromomeric regions of the chromosomal axis. 8 μ section of 13 week ovary. Magnification approximately 2,040 X.

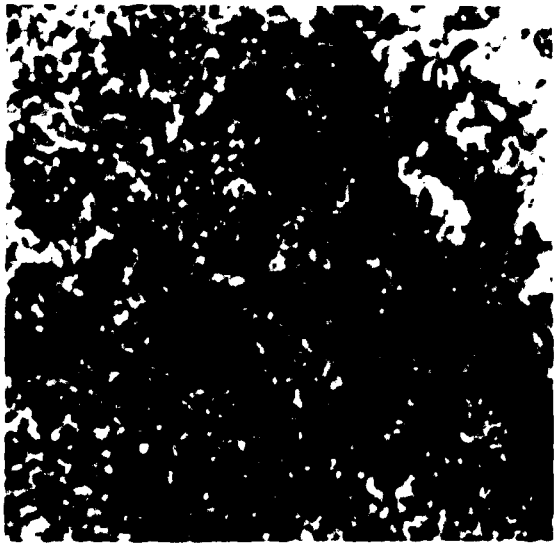
Fig. 47. Oocyte; 332 μ, nucleus 82 μ; showing S shaped lampbrush chromosome. Large arrow indicates the 'segmented' axis. Small arrow indicates the granulated lateral loops. 8 μ section of 13 week ovary. Magnification approximately 2,040 X.

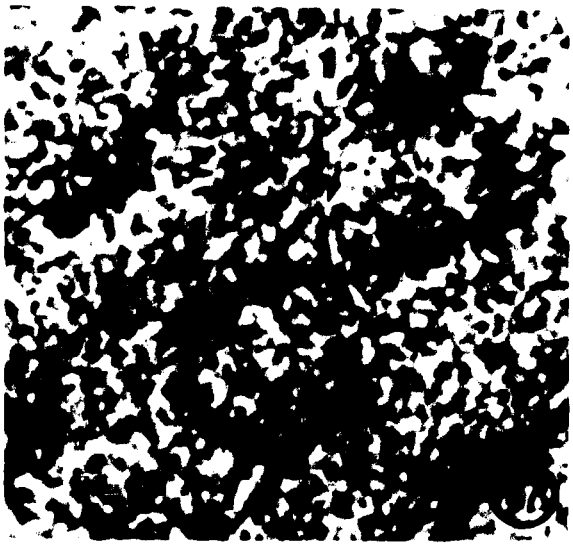
Fig. 48. Oocyte; 45 μ, nucleus 20 μ; showing some chromosomes in diplotene, others with condensed chromomeres. Arrow indicates incomplete double filament of chromosome. 8 μ section of hen ovary. Magnification approximately 1,730 X.

Fig. 49. Oocyte; 65 μ, nucleus 28 μ. Chromosomes sectioned across chromomeres were polar (a). The horizontal chromosomes were parallel to nuclear wall (b). Chromomere started becoming diffuse due to radiating bristle (c). Unlabelled arrows indicate a folded nuclear wall associated with the Balbiani body. 8 u section of hen ovary. Magnification approximately 1,730 X.

Fig. 50. Oocyte; 150 μ, nucleus 49 μ. Arrow indicates lampbrush chromosomal axis. Note folded nuclear wall and vivid ecto- (EC) and endo- (EN) nucleoplasmic area in the nucleus. 8 μ section of mature ovary. Magnification approximately 1,730 X.

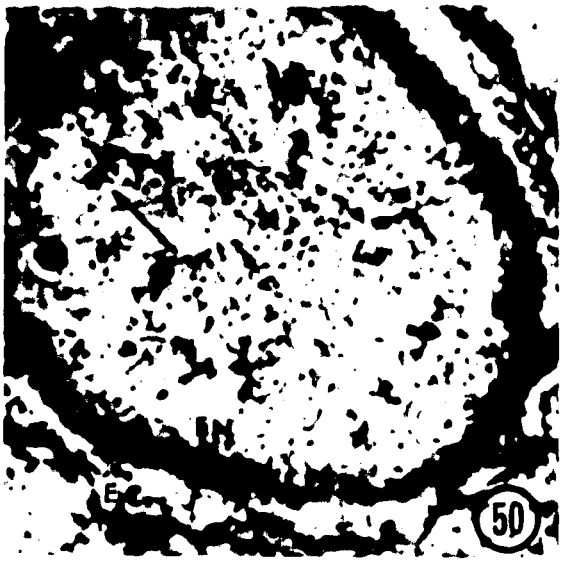
Fig. 51. A portion of 152 μ nucleus from 774 μ oocyte showing lampbrush chromosomes. Arrow indicates chromosome oriented parallel to nuclear wall. 8 μ section of laying hen ovary. Magnification approximately 600 X.






a
b


a
b


IN
E

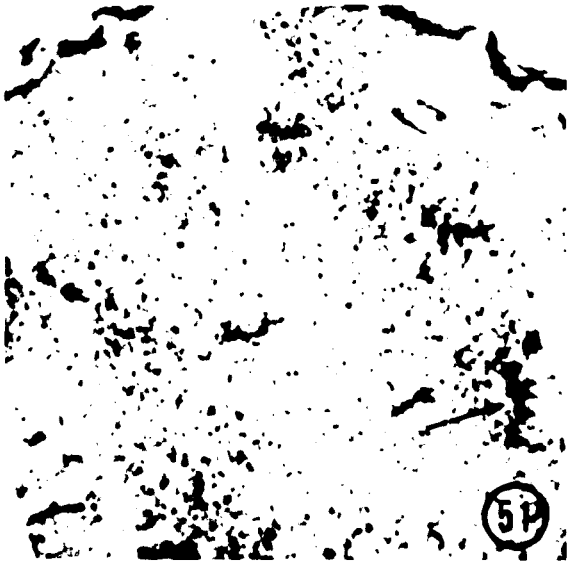


Fig. 52. A single lampbrush chromosome. Large arrow indicates distinct segmentation of the chromosome axis. Small arrow indicates a section through chromomere. 8 μ section of mature ovary containing nucleus, 150 μ; oocyte, 770 μ. Magnification approximately 1,730 X.

Fig. 53. A single lampbrush chromosome with lateral loops bearing granules (arrow) in single file. A large nucleolus (NL). 8 μ section of hen egg follicle containing nucleus, 175 μ; oocyte, 800 μ. Magnification approximately 1,740 X.

Fig. 54. Section through a small lampbrush chromosome. Arrow indicates a split chromomere. From 774 μ oocyte; containing 153 μ nucleus. Magnification approximately 1,740 X.

Figs. 55, 56. Section of 1.3 mm EF (oocyte 1 mm; nucleus 200 μ). Fig. 55. Arrow indicates chromomere involved in several loop formations. Fig. 56. Chromomeres condensed along the axis of chromosome. Arrow indicates one chromomere involved in forming stumpy loops on both sides. 8 μ section from hen egg follicle. Magnification approximately 1,740 X.

Fig. 57. Section of 2 mm EF, (oocyte 1.7 mm; nucleus 290 μ) showing single lampbrush chromosome with intact inter chromomeric axis (arrow). 8 μ section of laying hen. Magnification approximately 1,740 X.

Fig. 58. A section of 280 μ nucleus from 1.8 mm oocyte of hen, showing lampbrush chromosome about 40 μ long and chromomeres measuring .5 μ diameter. Arrow indicates one of the radiating loops. 8 μ section. Magnification approximately 1,740 X.

Fig. 59. Part of large 250 μ nucleus, from 5.0 mm oocyte (5.4 mm EF), of hen showing nucleoplasm laden with larger nucleoplasmic granules. Large arrow indicates granules superimposing chromosomal filaments. Small arrow indicates a knot-like object, probably condensed chromosomes. 8 μ section. Magnification approximately 1,740 X.

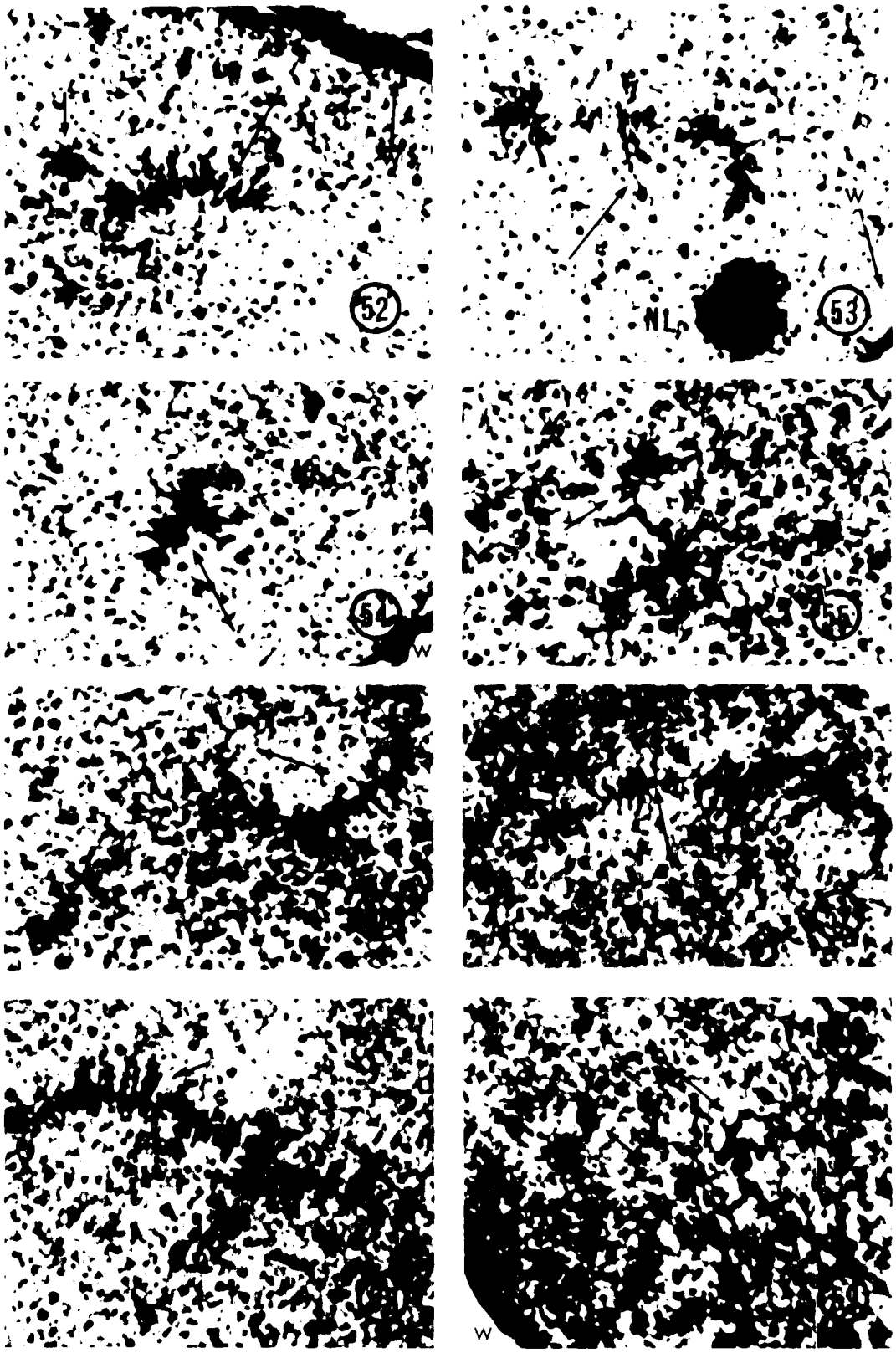

52
W
NL
53
54
W
55
W

Fig. 60. A 330 μ nucleus isolated from 1.5 mm EF mounted onto a glass slide and placed under glass coverslip. Arrow indicates chiasma type crosses in diplotene chromosomes a, b and c. Phase objective. Magnification approximately 800 X.

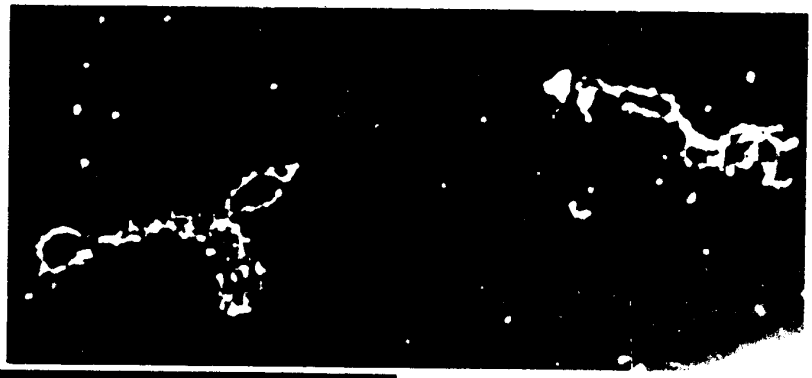
Fig. 61. Isolated lampbrush chromosomes in a 405 μ nucleus; 3 mm EF. Arrow indicates chromomeres on the main axis throwing fine barb-like lateral projections. Magnification approximately 1,500 X.

Fig. 62. A 400 μ nucleus isolated from 2.5 mm EF and mounted under coverslip. Magnification approximately 400 X.

Fig. 63. A 300 μ nucleus isolated from 6 mm EF mounted under coverslip. Peripheral nucleoli, classified as solid spheroid nucleoli (S). Magnification approximately 400 X.

Figs. 64, 65. A 300 μ nucleus isolated from 4 mm EF mounted under coverslip showing a network of mulberry type fibers bearing chromatic granules (CG). Magnification approximately 400 X.

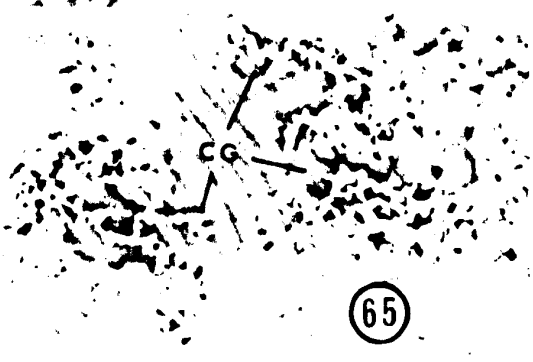
60



64

63

W



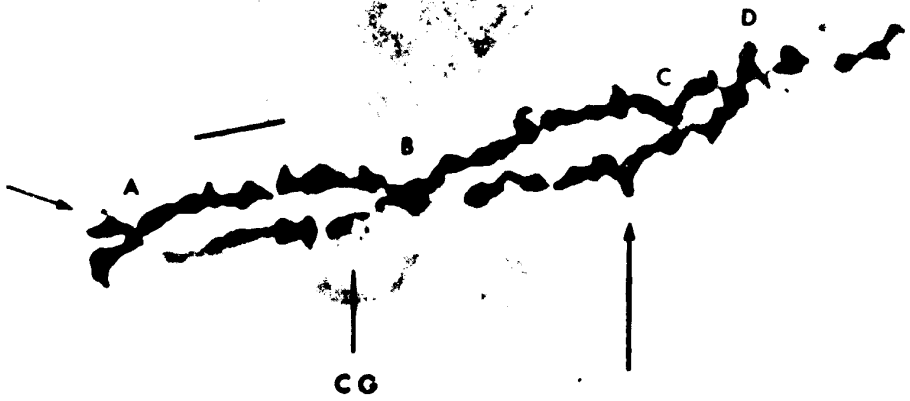
65

Fig. 66. Lampbrush chromosome isolated from 330 μ nucleus; 1 mm EF, of laying hen, showing bivalents crossing at A, B, C, D. Arrow indicates a large chromomere radiating four processes, two on each side. Note the chromomeres are of different dimensions and densities on the same chromatid. Phase. Magnification approximately 3,000 X.

Fig. 67. Lampbrush chromosome isolated from 333 μ nucleus; 1 mm EF, of laying hen showing chiasmatic crosses at A, B, C, D. Small arrow indicates point where one end of bivalent bifurcates into two chromatids, a large and small chromatic granule (CG) on the lower chromatid. The bar between A and B indicates alternating lighter chromomeres (large arrow) with stumpy lateral projections and denser angular chromomeres. Also note the bivalents tending to separate at chiasma C. Phase. Magnification approximately 3,000 X.

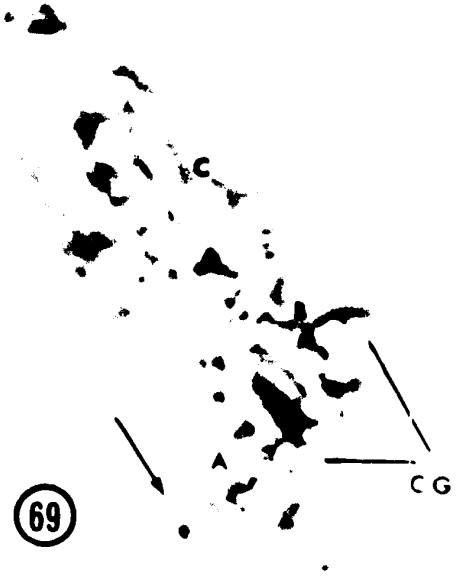
Figs. 68, 69. Lampbrush chromosomes. Fig. 68. Isolated from 334 μ nucleus, 1.5 mm EF. Note the granules (CG) and matrix material around the bivalent. Fig. 69. Isolated from 330 μ nucleus, 2.5 mm EF, showing the right arm bearing chromatic granules (CG). The arrow indicates that this chromatid appears to split into two sister chromatids. Phase. Magnification approximately 2,000 X.

66



67

68



69

Fig. 70. A lampbrush chromosome; attached to the nuclear wall (W) and isolated from 366 μ nucleus; 2.5 mm EF. Note lateral loops originating from chromomeres along the chromosome axis also covered by chromatic granules (CG). Note resemblance of the peripheral granules with CG studded on the lateral loops and with the chromomeres (CM). Also note chiasmata at A and B. Phase. Magnification approximately 800 X.

Fig. 71. Enlarged region (A-B) of Fig. 70 showing chiasmata A and B and the lateral loops (LL) on either sides of the lower arm. Phase. Magnification approximately 2,000 X.

Fig. 72. Same as above showing true chiasmata as the homologues are focused at the same level and the chromatic granules (white spheres). Phase. Magnification approximately 2,000 X.

Fig. 72 A. Right (A-B) of Fig. 72. showing the lateral loops (LL) and the axis of the chromosome bear granules of similar dimension and density as found dispersed in nucleoplasm. Phase. Magnification approximately 2,000 X.


70
CM
B
C
A
CG
CM
W
71
A
72
CG
A
A

Fig. 73. A monovalent of a lampbrush chromosome isolated from the same nucleus as Fig. 70. showing chromatic granules (CG) along the main axis of the chromosome as well as along the lateral loops (LL). The arrow indicates the end of the chromatid which appears bifurcated. Phase. Magnification approximately 800 X.

Fig. 74. Region X-Y of Fig. 73. enlarged. Arrows indicate the attachment of chromatic granules along the entire axis of the chromosome. Phase. Magnification approximately 2,000 X.

Fig. 75. Region X-Y of Fig. 73. showing that the lateral loops (LL) have similar beaded appearance as chromomeres (CH) on the main axis of chromosome. The chromatic granules (CG) attached to lateral loops are similar to those attached to the main axis of chromosome. Phase. Magnification approximately 2,000 X.

73

74

75

Fig. 76. Lumpy loop chromosome element isolated from 333 μ nucleus; 2 mm EF. Arrows indicate where the beaded main axis has branched into a number of later loops. Lateral loops merged into lumpy region (L). Phase. Magnification approximately 2,000 X.

Fig. 77. Lumpy chromosome element isolated from 366 μ nucleus; 3 mm EF. Note one end forming a lumpy mass (L) while the other end involved in unfolding into fibers of finer dimension (F). Phase. Magnification approximately 1,000 X.

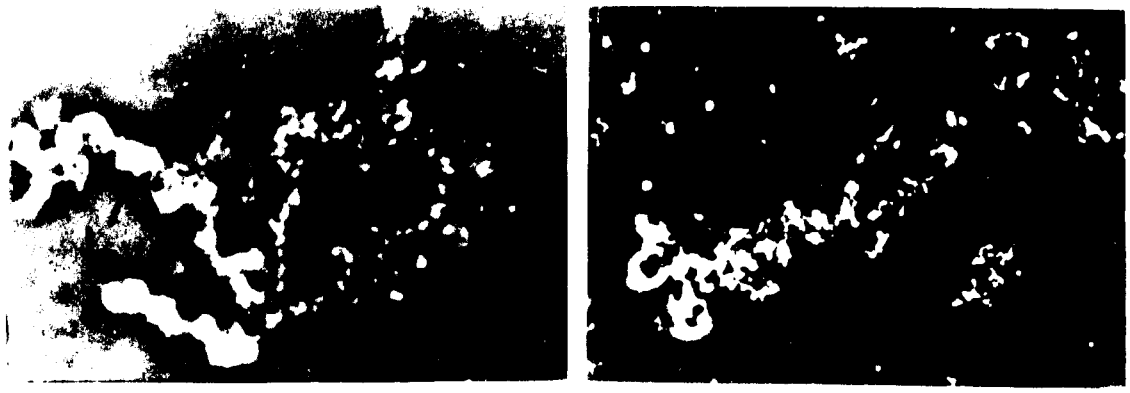
Figs. 78, 79. Chromosome element isolated from the same nucleus as Fig. 77. showing denser and more refractile fibers (CM) and other diffused and finer fibers (F) which resemble lateral loops (LL). Fig. 79. Chromatids give appearance of bridges (CM). Phase. Magnification approximately 1,000 X.

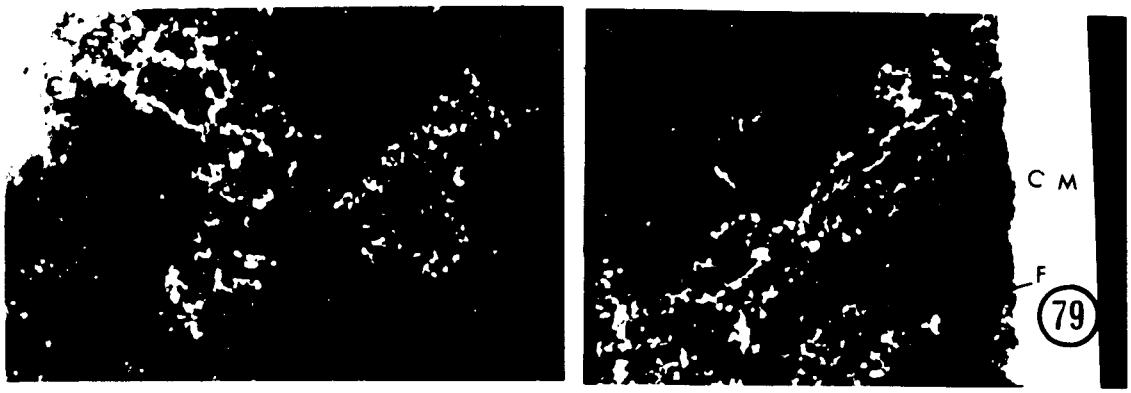
Figs. 80 A, 80. Mulberry type chromosome elements. Fig. 80 A. Isolated from 333 μ nucleus; 4 mm EF, elements resting on nuclear wall. Fig. 80. Isolated from 350 μ nucleus; 4 mm EF. Chromosomal elements rest on formver film of electron microscope grid. Phase. Magnification approximately 200 X.

Figs. 81 A, 81. Mulberry type chromosome elements isolated from 323 μ nucleus; 3 mm EF showing that because of chromomeres (CM) the main axis and lateral branches (LL) have similar beaded appearance. Phase. Magnification approximately 1,000 X.

Figs. 82 A, 82. Mulberry type chromosome elements; Fig. 82 A. Isolated from 336 μ nucleus; 4 mm EF, showing chiasmatic arms bearing chromomeres and chromatic granules (CG). Fig. 82. Isolated from 340 μ nucleus; 2 mm EF, showing two arms covered by nucleoplasmic matrix, chromatic granules (CG) and peripheral granules. Phase. Magnification approximately 1,000 X.

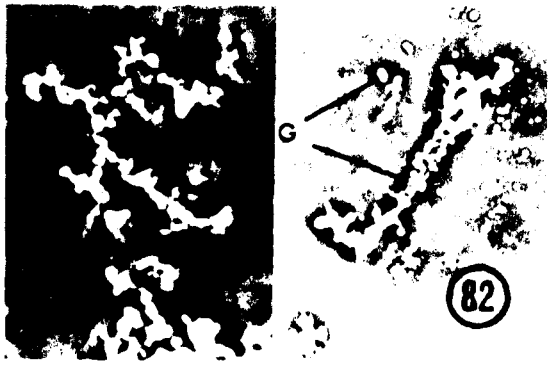
Fig. 83. Mulberry type chromosome element stained by Fast green, isolated from 368 μ nucleus; 5.5 mm EF. Note stained chromomere (CH) along axis. Magnification approximately 200 X.




C M
F
79


80


M
L
81


G
82

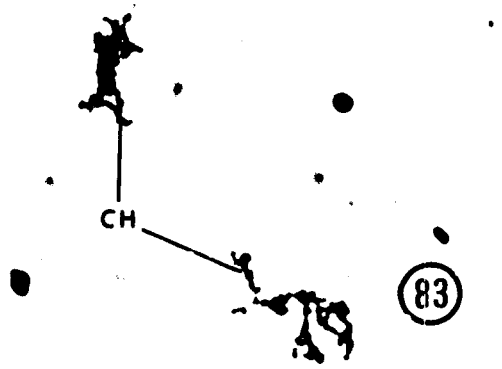

CH
83

Fig. 84. Large mulberry type chromosomal elements isolated from 425 μ nucleus; 6.6 mm EF. Small arrow indicates the axis where the 2000 Å basic strand bearing about 3000 Å chromomere (CH) is free from chromatic granules (CG). The large arrow indicates a place where the basic strand is beyond the limit of resolution. Phase. Magnification approximately 2,000 X.

Fig. 85. Large mulberry type chromosomal element isolated from 336 μ nucleus; 5.5 mm EF. Two inward arrows show a long straight 2000 Å thick fiber studded with 3000 Å chromomeres (CH) and chromatic nuclei (CG). The forked arrows indicate a bridge like bifurcation of the same fiber. Phase. Magnification approximately 2,000 X.

Fig. 86. Mulberry type chromosomal element isolated from 366 μ nucleus; 2 mm EF. Note the basic chromosomal strand (CM) studded with chromomeres (CH) and chromatic nucleoli (CG). Phase. Magnification approximately 2,000 X.

CH
84

Fig. 87. Nucleolar chromosome elements from 366 μ nucleus; 6.5 mm EF. Note the axis bearing similar size chromatic nucleoli (CG). Phase. Magnification approximately 1,000 X.

Fig. 88. Nucleolar chromosome elements from 330 μ nucleus; 2 mm EF. Branched axis studded with chromatic nucleoli (CG), about 2 μ. Phase. Magnification approximately 1,000 X.

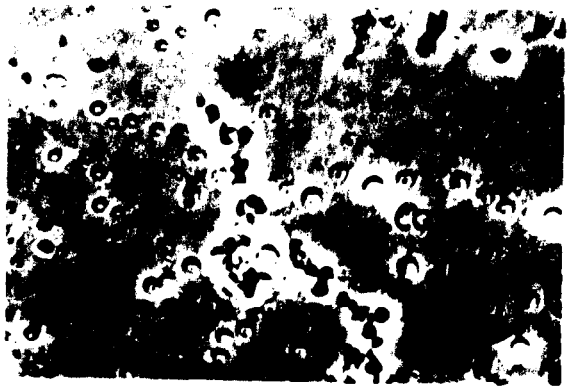
Fig. 89. Nucleolar chromosome elements from 400 μ nucleus; 2.5 mm EF. Note lumpy, ribbon like axis bearing chromatic nucleoli ranging from 1 - 5 μ dia. Phase. Magnification approximately 1,000 X.

Fig. 90. Nucleolar bearing chromosome elements from 366 μ nucleus; 3 mm EF. Note branched axis, lateral loops (LL) and 1 - 5 μ nucleoli (CG) are heavily stained. Clear surface of nucleoli is due to reflection. Hematoxylin stained. Magnification approximately 1,000 X.

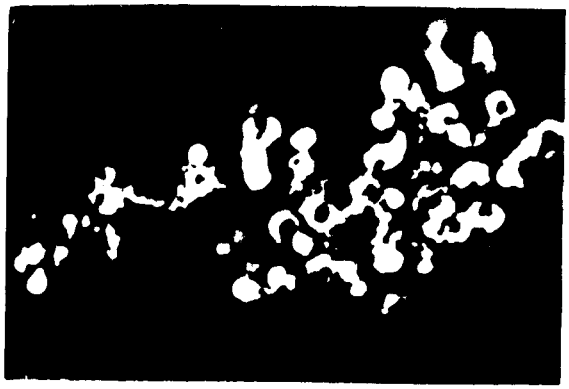
Figs. 91, 92. Lampbrush chromosome freshly isolated from 366 μ nucleus; 2 mm EF. Note lumpy bilateral loops and chromatic granules. Fig. 91. Before treatment. Fig. 92. After adding a quantity of crystalline trypsin. The arrow indicates the axis shrank

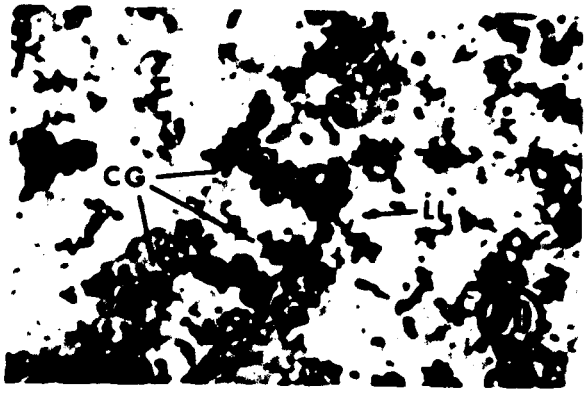
but became clear of granules. Phase. Magnification approximately 1,000 X.

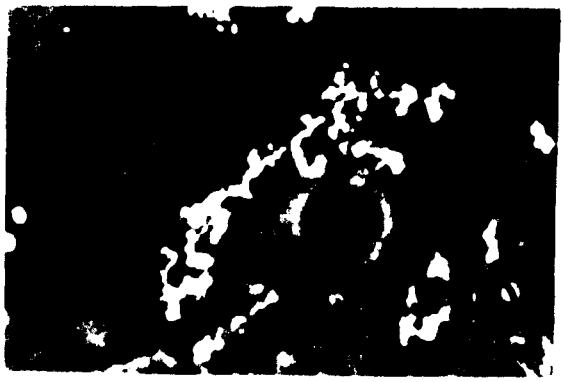
Figs. 93, 94. Small mulberry type chromosome element from 366 μ nucleus; 4 mm EF, showing undulating axis. Fig. 93. Before treatment. Fig. 94. After treating 3.5 min with pronase. The basic fiber with chromomeric (CH) axis became clear. Phase. Magnification approximately 1,000 X.

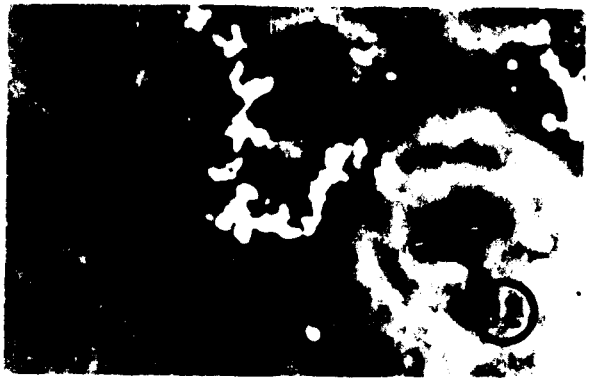


88









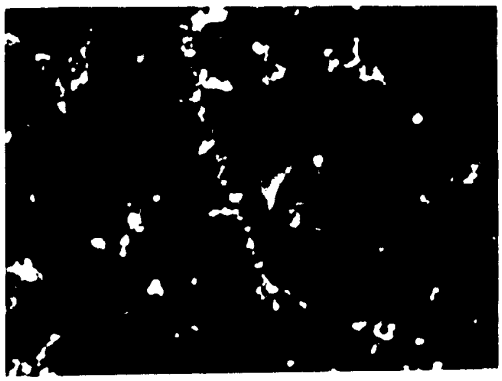
93

94

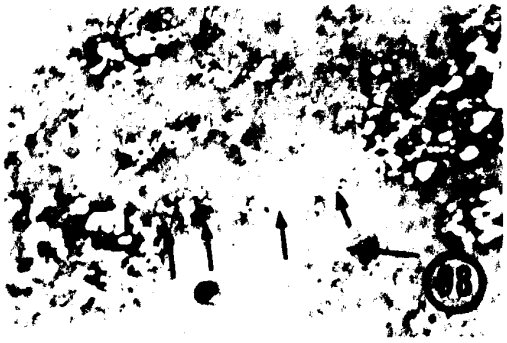
Figs. 95, 96. Mulberry type chromosome element from 334 μ nucleus; 1.5 mm EF. Fig. 95. Before treatment. Note chromomeric axis (X-Y) and the beaded lateral loops (LL). Fig. 96. After 20 min of pronase (.25 mg/ml) and 45 min of DNase action. The main axis and loops were sliced transversely. Only chromatic granules and chromomeric spots are left in linear order. Arrows indicate position of axis and loop. Phase. Magnification approximately 1,000 X.

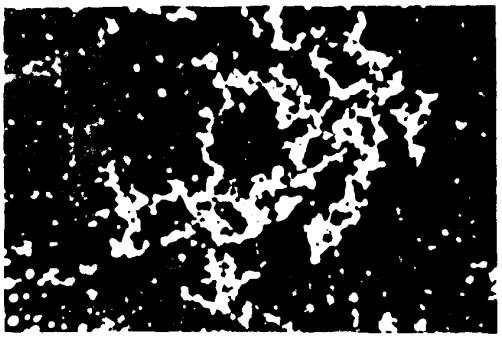
Figs. 97, 98. Large mulberry type chromosome element from the Fig. 95 source. Fig. 97. Arrows indicate the continuity of the axis after 20 min pronase action. Fig. 98. Arrows indicate points of breakage after 45 minutes of DNase action. Phase. Magnification approximately 1,000 X.

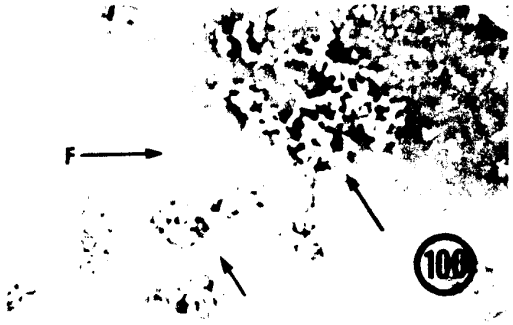
Figs. 99, 100, 101. Lumpy looped chromosome elements from same nucleus as in Fig. 95. Arrows indicate thick, bright, lumpy region and fine diffused fibers (F). Fig. 99. Before treatment. Fig. 100. After 10 min treatment of pronase. Fig. 101. After 35 min of DNase treatment. Phase. Magnification approximately 1,000 X.




Y
96





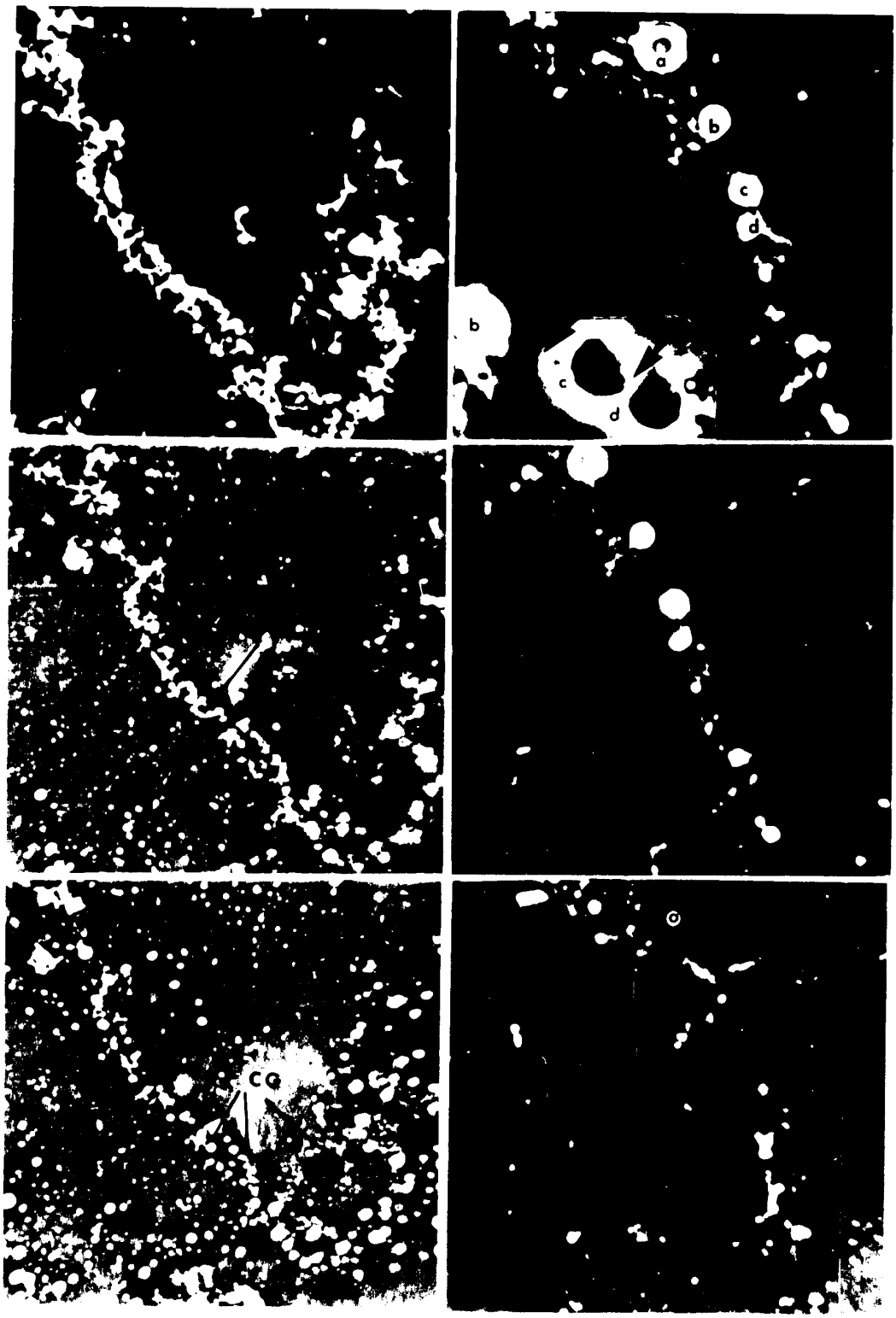

F
106


F
101

Figs. 102, 103, 104. Lampbrush chromosome isolated from 334 μ nucleus; 1.5 mm EF, showing intact chromomeric axis (arrow), and chromatic granules (CG) placed more towards extremity. Fig. 102. After treatment with pronase for 20 min. The granules and chromomeres became bright and more distinct. Fig. 103. After DNase treatment for 45 min. The arrows indicate where the longitudinal axis was sliced transversely at inter chromomeric regions. Fig. 104. After treatment with RNase. Note a complete disassociation of inter chromomeric regions and detachment of the chromatic granules (CG). Phase. Magnification approximately 2,000 X.

Figs. 105, 106, 107. Freshly isolated nucleolar lampbrush chromosomes from 400 μ nucleus; 6.2 mm EF. Fig. 105. Before treatment. Note chromosomal axis connecting chromatic nucleoli (a, b, c, d) in the middle and smaller nucleoli clustered at the extremity. Fig. 105A. Enlarged area of Fig. 105. Magnification approximately 3,000 X. Arrows show chromosomal axis. Note the unattached beaded ring type nucleoli (NL) lying close to the chromosome. Fig. 106. After treatment of DNase for five hours. Arrows indicate no effect of enzyme on chromosomal axis. Fig. 107. Five min after adding 2 drops of pronase. The arrows indicate where the axis was severed. Lateral loops

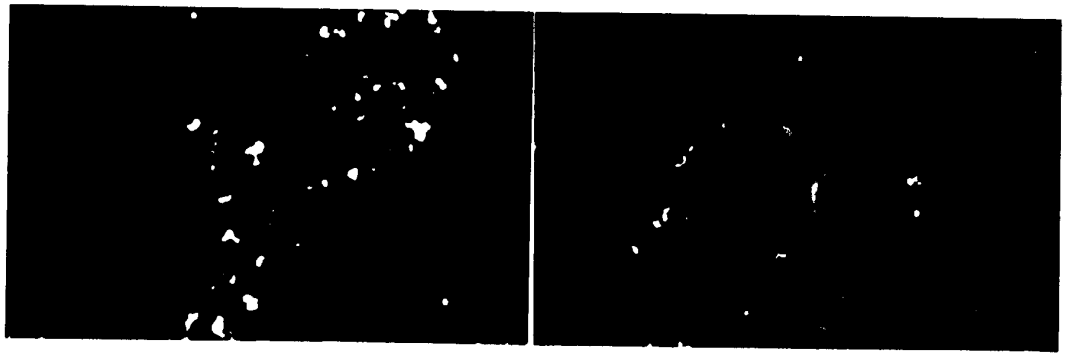
and detached nucleoli (a, b, c, d) were reduced in dia and also beaded ring nucleoli (X) disassociated. Phase. Magnification approximately 2,000 X.

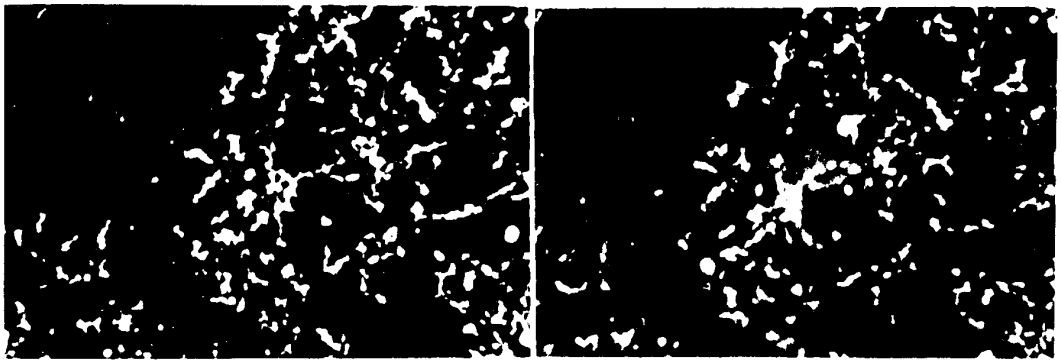

a
b
c
d
b
c
d
CG

Figs. 108, 109, 110, 111. Mulberry type branched chromosome elements from 368 μ nucleus; 3 mm EF. Figs. 108 and 110 show no change after 1 hr DNase action. Fig. 109 is Fig. 108 after 4 minutes exposure to two drops of pronase. Fig. 111 is Fig. 110 after 4 minutes exposure to one drop of pronase. Arrows indicate cite of action of enzyme which severed the axis. Phase. Magnification approximately 1,000 X.

Figs. 112, 113. Mulberry type chromosome elements bearing nucleoli (CG) isolated from 400 μ nucleus; 5.5 mm EF. Fig. 112. Treated for 1 1/2 hours with DNase and no change observed. Fig. 113. is Fig. 112. after treating with pronase. Note that most of the fibrillar elements were sliced transversely. Phase. Magnification approximately 1,000 X.

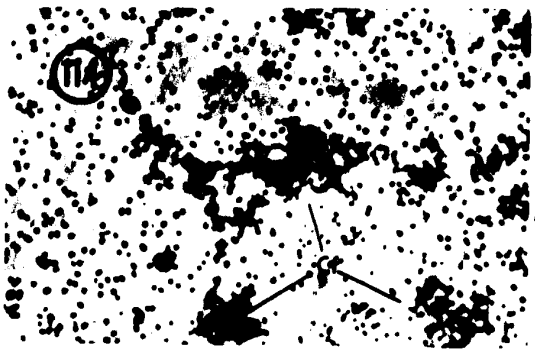
Figs. 114, 115. Mulberry type chromosome elements (CF) exposed for 4 weeks to tritiated Actinomycin D. Fig. 114. Isolated from 360 μ, 2.5 mm EF. Fig. 115. Isolated from 400 μ nucleus, 4 mm EF. Phase. Magnification approximately 1,000 X.






112


113


114


115

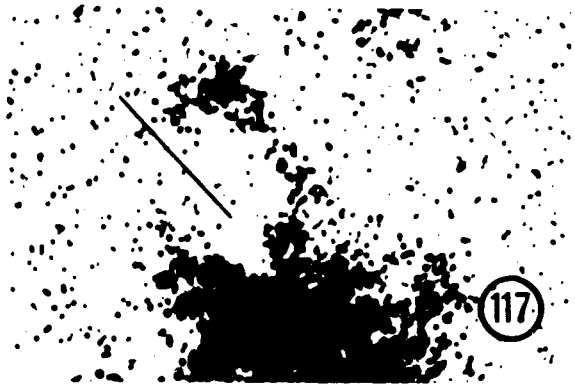
Figs. 116, 117. Unspread chromosomal materials freshly isolated from 333 μ nucleus; 3 mm EF. Fig. 116. Before autoradiography; Fig. 117. after autoradiography. Phase. Magnification approximately 400 X.

Figs. 118, 119. Chromosome elements attached to one end of nuclear membrane (W) isolated from 400 μ nucleus; 2.5 mm EF. Fig. 118. Before autoradiography; Fig. 119. after autoradiography. Magnification approximately 400 X.

Figs. 120, 121. Mulberry type chromosome elements isolated from 400 μ nucleus; 6 mm EF. Fig. 120. Before autoradiography; Fig. 121. after autoradiography. Phase. Magnification approximately 1,000 X.

Figs. 122, 123. Nucleolar lampbrush chromosome isolated from 400 μ nucleus; 2.5 EF. Fig. 122. Before autoradiography; Fig. 123. after autoradiography. Note chromatic nucleoli (CG) did not show grains. Phase. Magnification approximately 1,000 X.

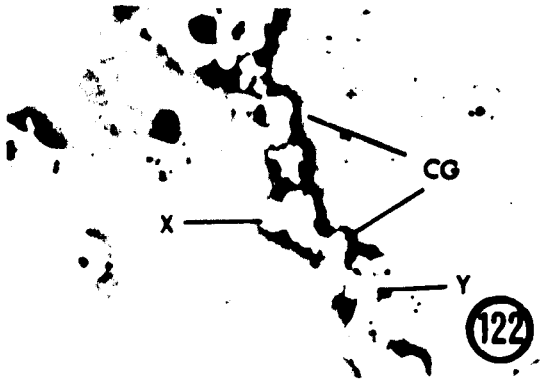
116



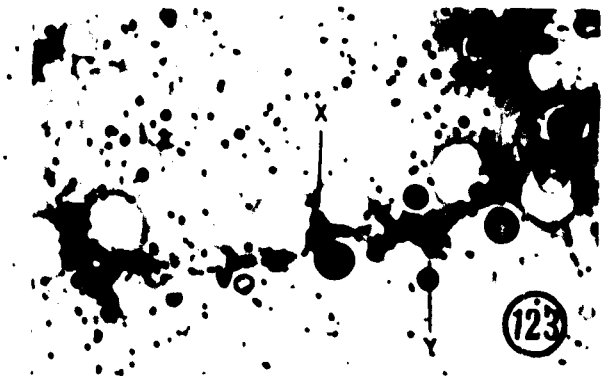
117

120

121



122



123

Fig. 124. Whole mount preparation of lampbrush chromosomes isolated from 360 μ nucleus; 3 mm EF, showing two basic fibers (bars) constituting the framework of each chromosome. Arrows indicate where the fibers became exposed and measured 450 Å. EM 100. Magnification approximately 7,200 X.

Fig. 125. Chromosome elements isolated from 300 μ nucleus; 2 mm EF. Arrows indicate basic fibres measuring about 250 Å. EM 100. Magnification approximately 80,000 X.


124
125

Fig. 126. Fig. 124 magnified, showing a central region (CH) from which microfibrils radiated. The arrows indicate areas of the microfils which were less than 400 Å. EM 100. Magnification approximately 9,600 X.

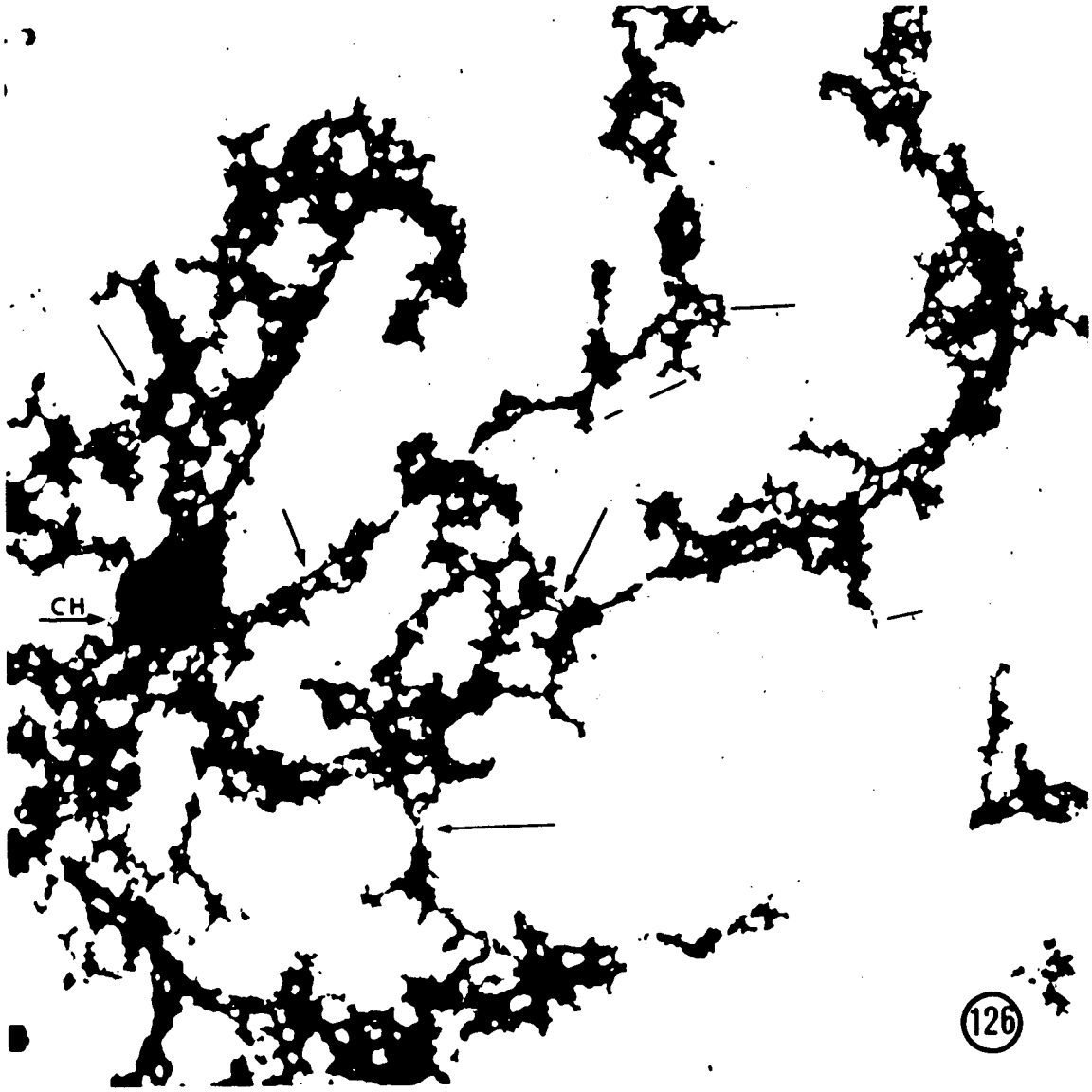
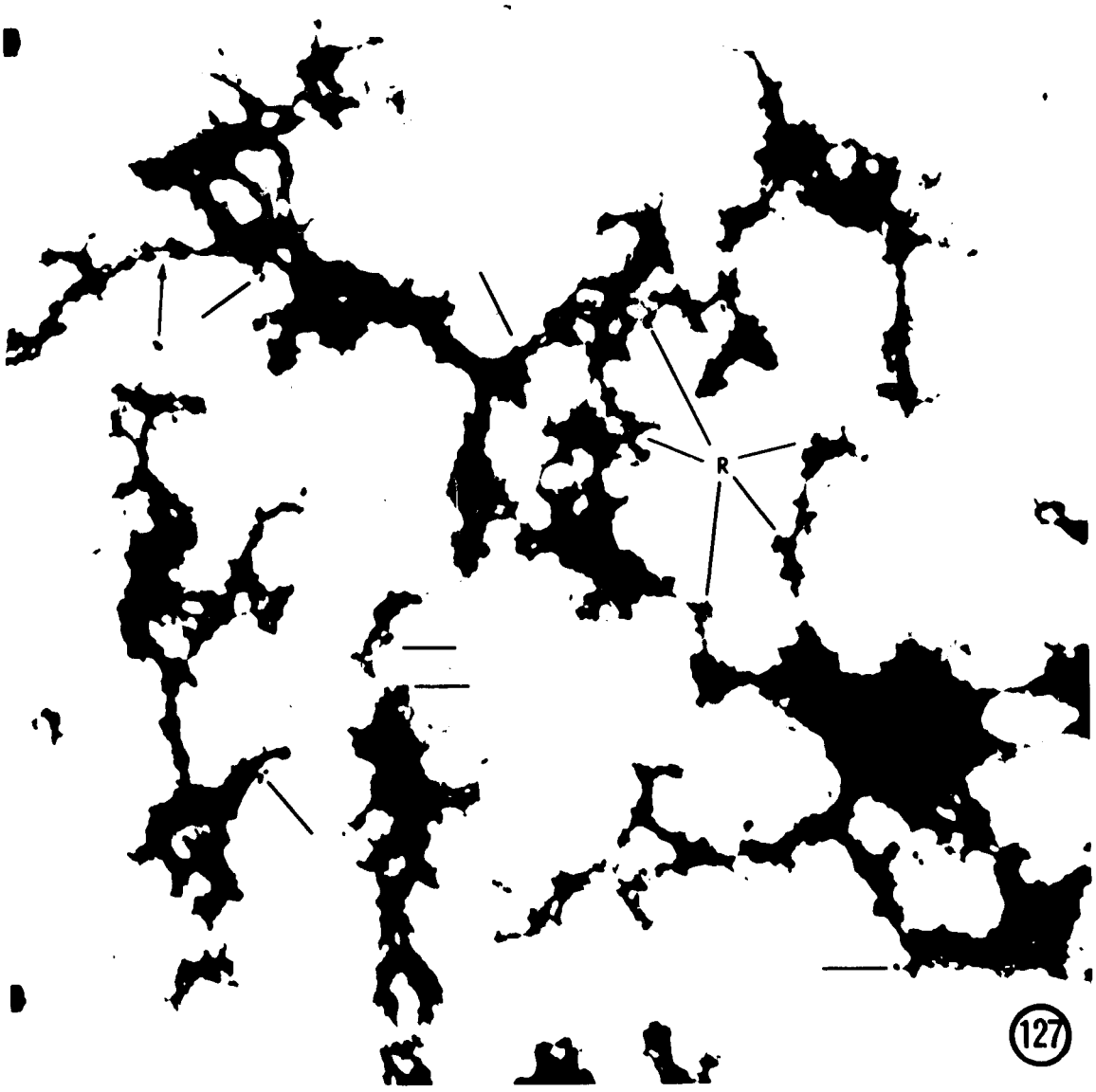
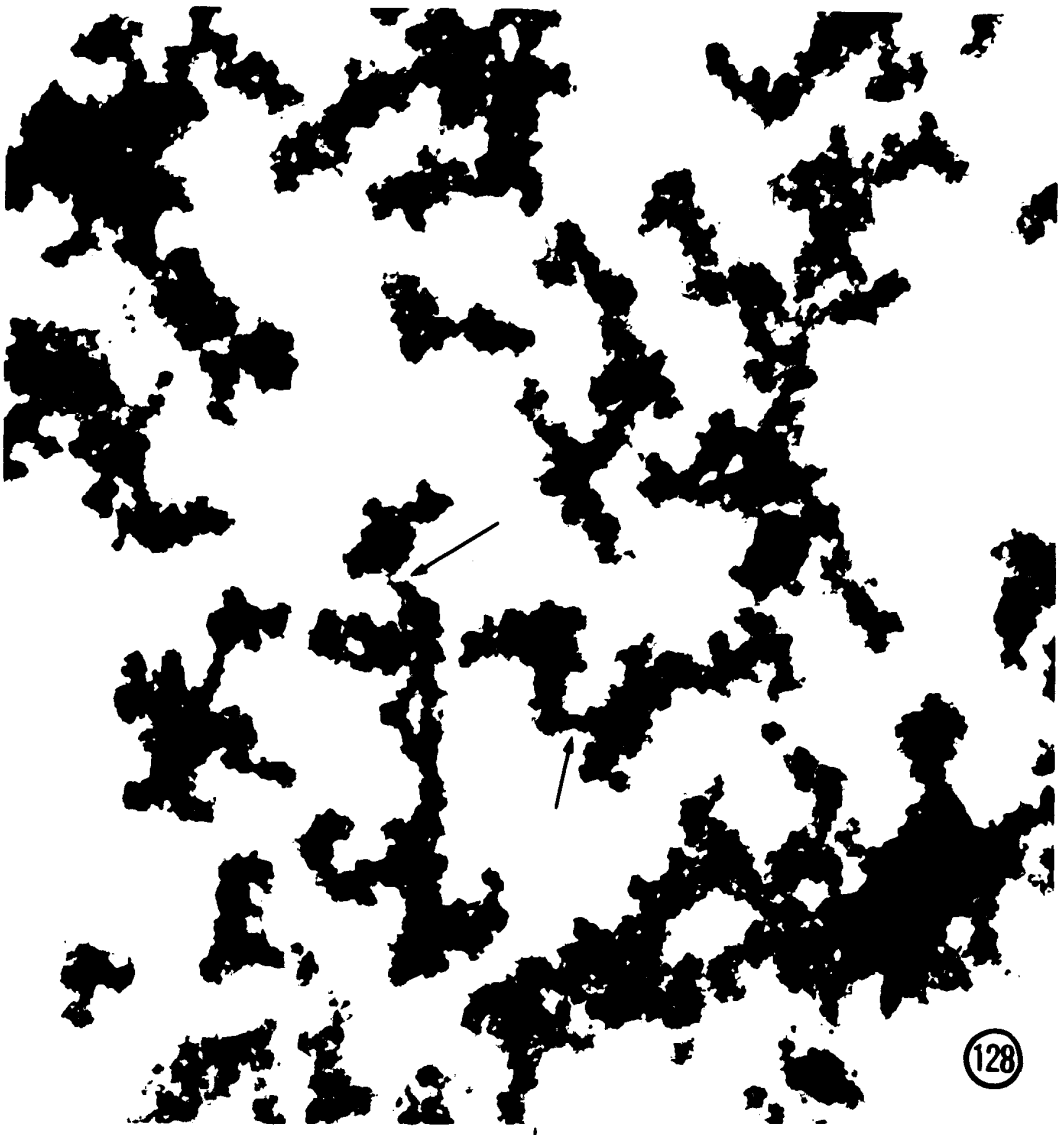

CH
126

Fig. 127. Same as 124., magnified; The arrow indicates two basic microfibrils, each 250-300 Å, involved with the formation of the central structure. Both appeared to be impregnated with dense particles (bars and R) of 300 Å. Uranyl acetate stained. EM 100. Magnification approximately 21,600 X.


R

Figs. 128, 129. Chromosome elements isolated from 300 μ nucleus; 3 mm EF, showing mulberry type elements. Fig. 128. Element appeared independent and composed of interphase like chromatin fiberils. Arrows indicate places measured. EM 200. Magnification approximately 88,200 X. Fig. 129. Showing dichotonmy of fibrillar elements, a fine (F) and a thicker (T) fibrillar strand which were measured at places shown by arrows. EM 200. Magnification approximately 34,000 X.



128

129

Figs. 130, 131. Nucleolar lampbrush chromosome elements isolated from 333 μ nucleus; 1.7 mm EF fixed in glutraldehyde and osmium fumes, stained in 2% uranyl acetate. Fig. 130. Note chromatic granules (CG) involved with the two chromosomal fibers, thicker (T) and finer (F). Arrow indicates the spot where fine fiber were measured. Fig. 131. A portion of nucleolar chromosome which appears to be composed of two coiling strands indicated by two arrows. EM 100. Magnification approximately 32,400 X.


T
CG
F
130
131

Fig. 132. Nucleolar chromosome element from the same source as Fig. 130. Ribbon like coiled chromosomal basic strand (T) attached to larger nucleoli (GG). Small chromatic granules appear to be budding from circumference of nucleoli. EM 100. Magnification approximately 36,000 X.

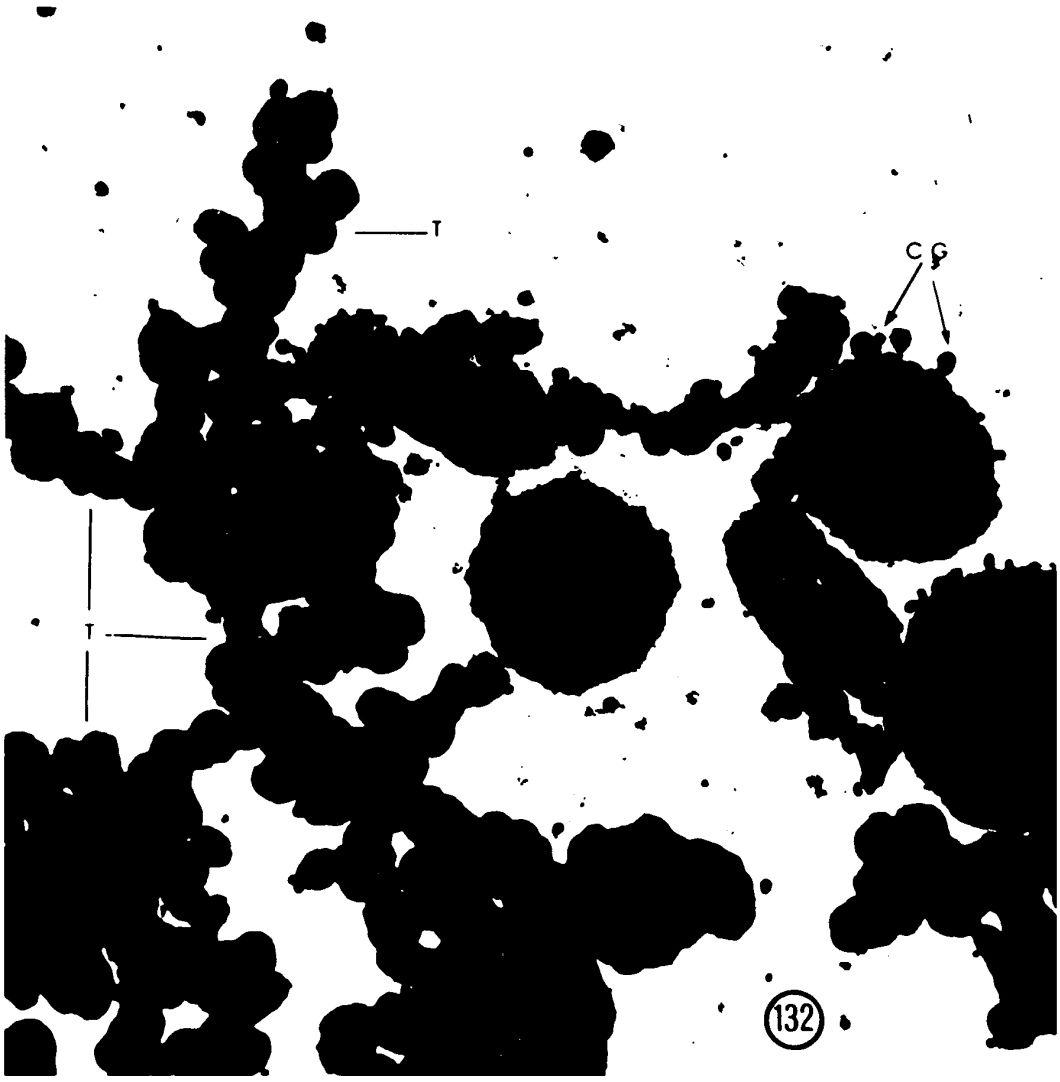

T
T
C
C
132

Fig. 133. Nucleolar lampbrush chromosome elements from the the same source as Fig. 130. Note large and small nucleoli (NL) attached to two 250 Å chromosomal basic fibers (F and arrows) which form the main strand of lampbrush chromosome (T). EM 100. Magnification approximately 32,400 X.

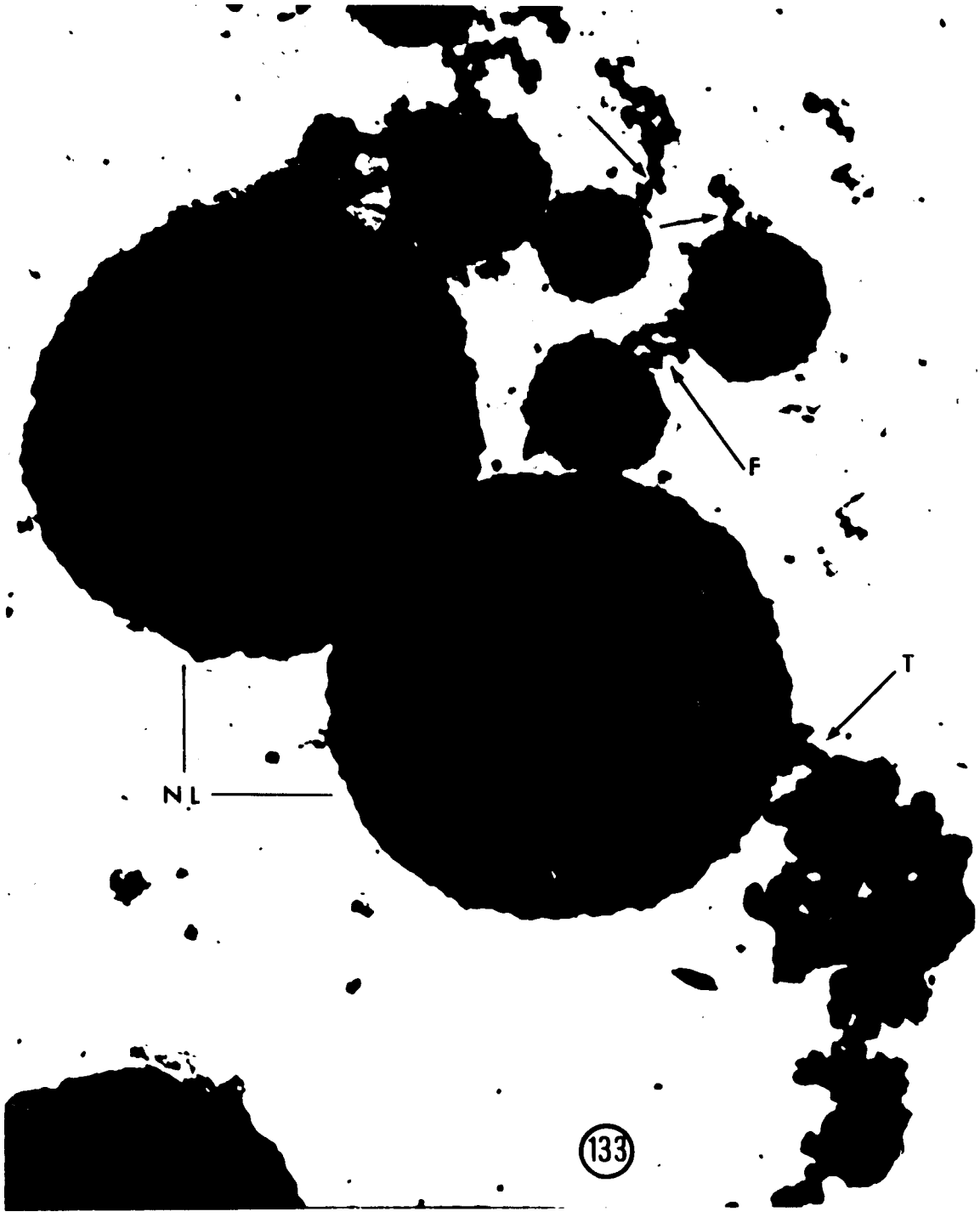
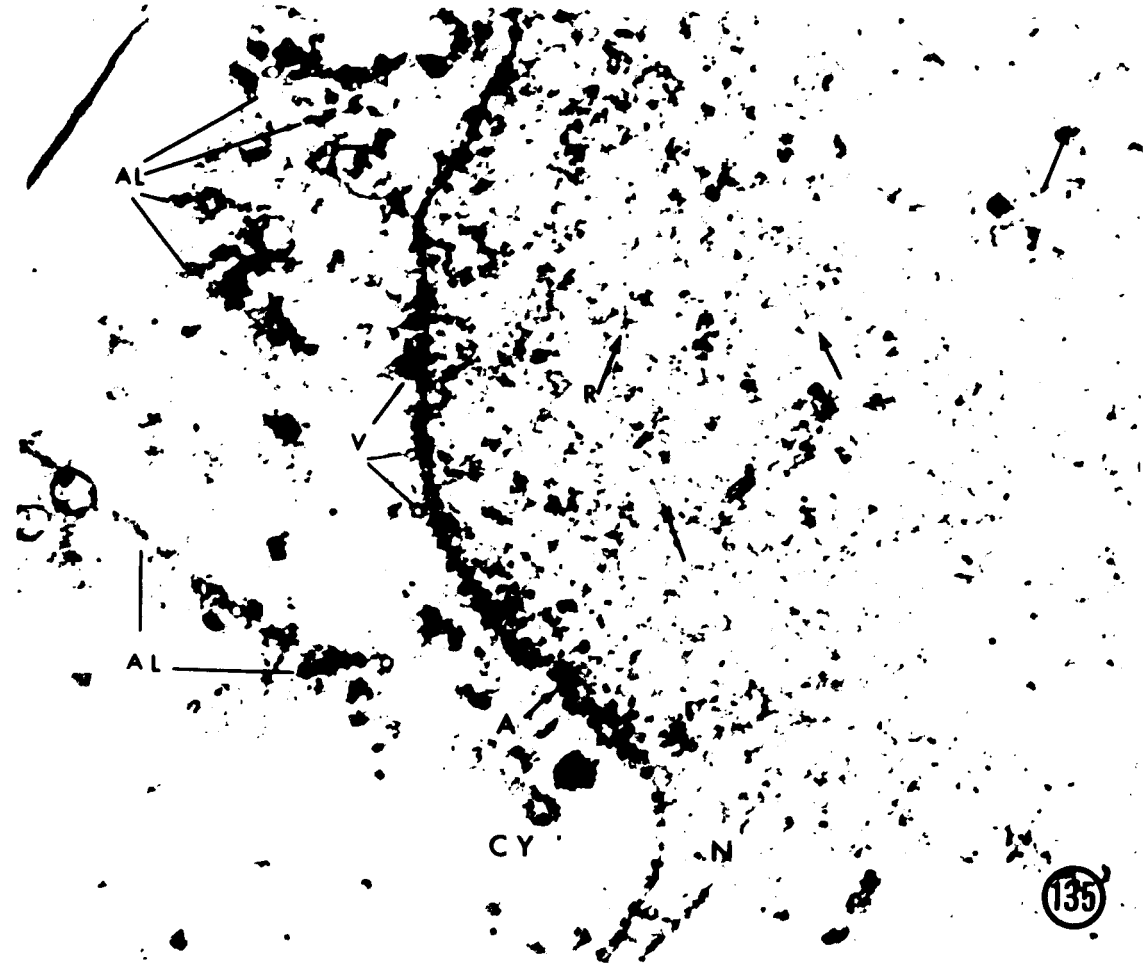

F
T
NL
133

Fig. 134. Section of 360 μ isolated nucleus showing reticulum of 200 Å, thick fibers (arrows), and condensations a, b, c, d, e, and f. Note a branched axis of the fibrillar material (X-Y), also annular rings (A) at the nuclear membrane. Particles of 300-400 Å (R) are also present in chain or groups. EM 100. Magnification approximately 36,000 X.


a
b
c
d
e
f
Y
R
A
X
CY
W
N
R
134

Figs. 135, 136. Section of isolated 2 mm EF, showing nucleo cytoplasmic region. Note the nucleus consists of a network of microfibrils (arrow) forming microvesicles. Some fibers bear dense particles in a row (R). Fig. 135. Shows that the nuclear wall has multiple annular rings at places (A). Nuclear wall involved with annulate lamilae (AL) or circular (v) vesicles of the cytoplasm. EM 100. Magnification approximately 9,000 X. Fig. 136. Nucleocytoplasmic region shows vesiculated nature of annular material (AM). EM 100. Magnification approximately 42,000 X.


AL
V
R
AL
A
CY
N
135

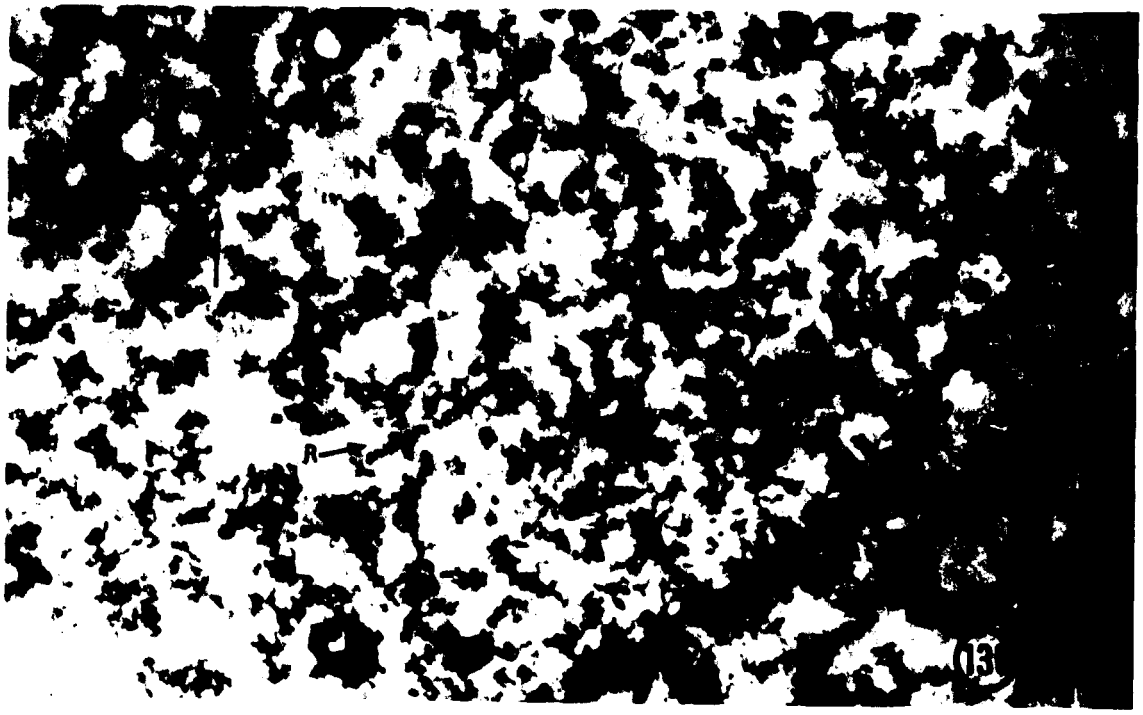
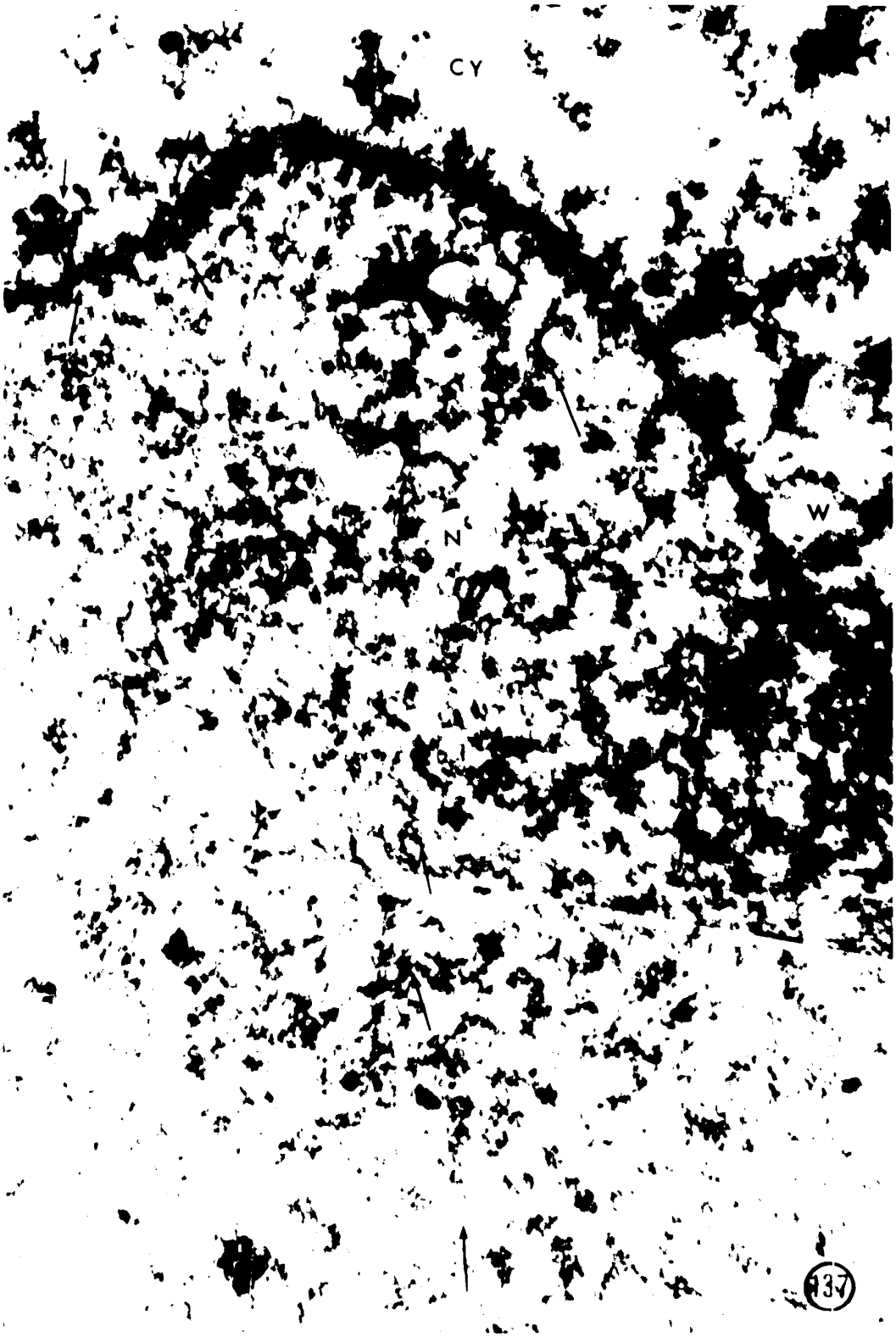

N
R
03

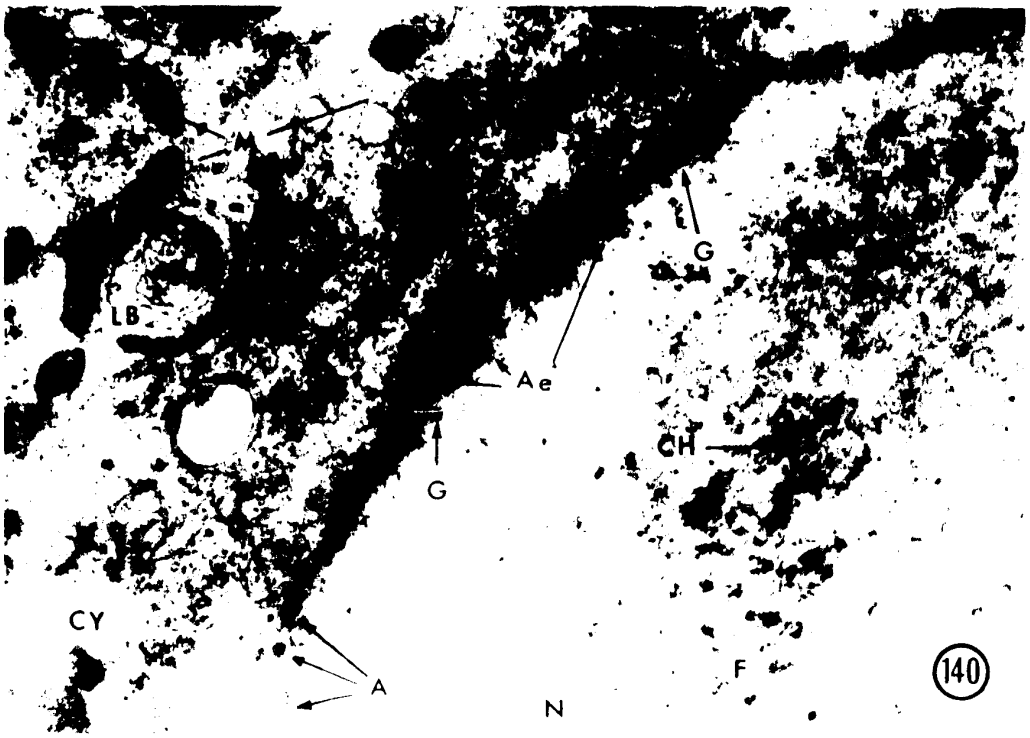
Fig. 137. Nucleocytoplasmic region of 2 mm egg follicle showing nuclear reticulum consisting of microfibrils forming microvesicles (arrows). Pointing arrows indicate microvesicles which appear to be crossing the nuclear wall (W). EM 100. Magnification approximately 21,600 X.


CY
W
N
137

Figs. 138, 139. Nucleocytoplasmic regions of 2.5 mm EF. Fig. 138. Arrows indicate microfibrils involved in forming nuclear microvesicles (mv). Fibers bear dense particles in single row (R). EM 100. Magnification approximately 25,000 X. Fig. 139. Passage of microvesicles across the envelope is also visible (opposite arrows). Other arrows indicate microvesicles. EM 100. Magnification approximately 21,000 X.


CY
MV
R
N
W
CY
N

Figs. 140, 141. Ovarian tissue (7 week) showing nucleocytoplasmic section containing 10 μ nucleus, 18 μ oocyte. Fig. 140. Note mitochondrial cloud (M), multiple vesicles (MV) of the cytoplasm including lining body (LB). Nuclear envelope shows annular rings (A). Some rings contained central granules (G), other rings are empty (Ae). EM 200. Magnification approximately 21,600 X. Fig. 141. The chromosomes appear to be composed of fibrillar structure (F) which at places demonstrates chromomeric condensation (CH). EM 200. Magnification approximately 30,000 X.



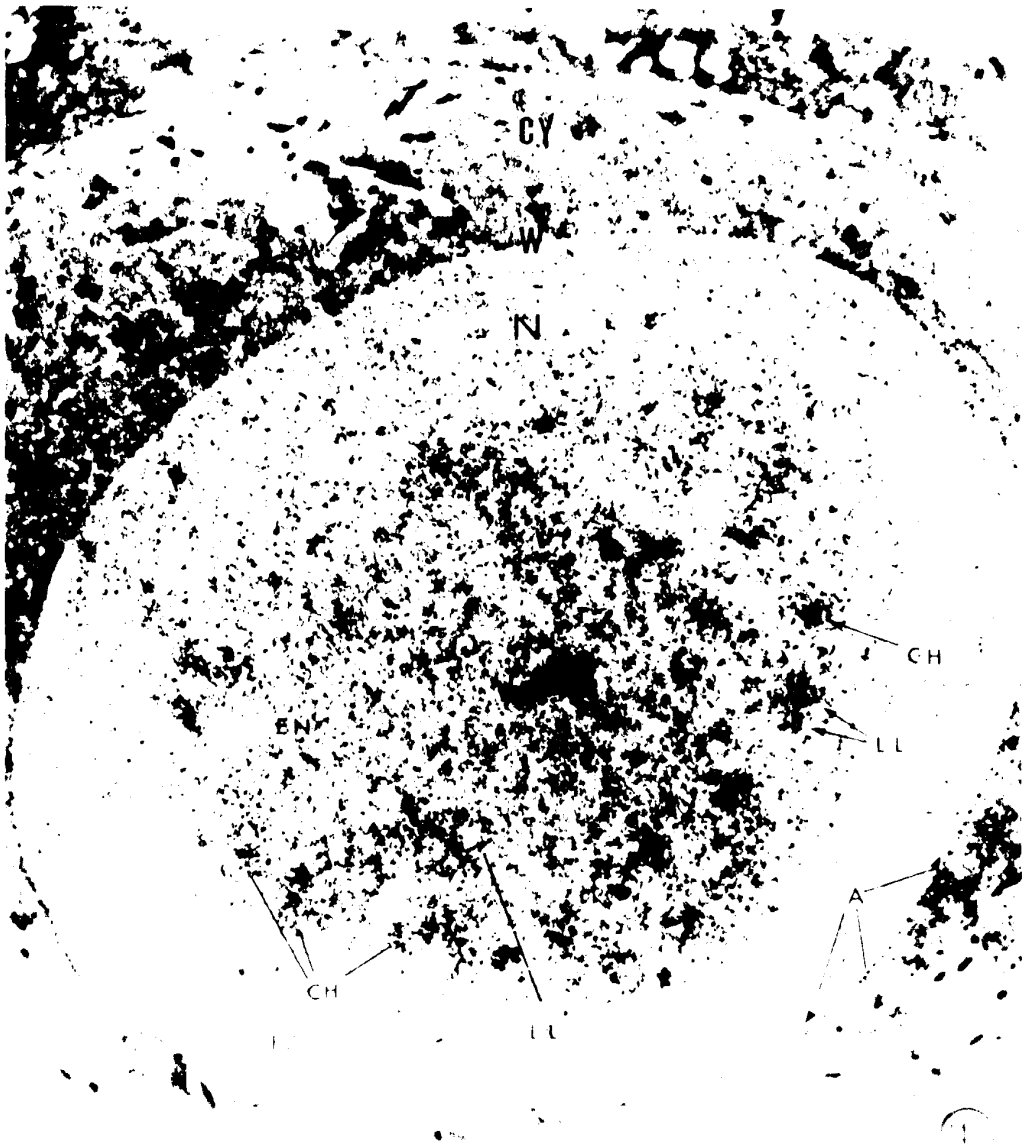
140

141

Fig. 142. Oblique section of ovarian tissue (7 week) containing 13 μ nucleus; 21 μ oocyte. Note 2, 3 rows of annular rings (A), some of which contained electron dense material, others empty (Ae). The chromosome microfibrils (F) dispersed in nucleus. Black spot is an artifact. EM 100. Magnification approximately 14,400 X.


CY
Ae
N
F
A
142

Fig. 143. Tangential section of 29 μ oocyte; 20 μ nucleus, contained in 7 week ovarian tissue showing nuclear wall (W). Annular rings (A) are in a single row along the circumference of nucleus. Note ecto-nucleoplasm (EC) and endonucleoplasm (EN). Centralized chromosomal fibrils, and chromomeric spots (CH) appear to extend into lateral projections (LL) and are arranged towards periphery of endo-nucleoplasm. EM 200. Magnification approximately 7,000 X.


CY
W
N
CH
LL
BN
CH
LL
A

MORPHOLOGY OF ISOLATED NUCLEOLI

Literature Review

During isolation of lampbrush chromosomes from oocyte nuclei, not only the chromosomal elements but also several heteromorphic objects (nucleoli) were observed. Although it is generally recognized that maturing oocytes of amphibians, fish and insects contain multiple nucleoli, there are no reports in the literature indicating whether this situation exists in chickens.

Brachet (1942) reported that a large number of nucleoli were directly associated with intensive synthetic activity of the cell. Not only has a relationship between the nucleolus and the synthesis of RNA been established (Perry 1962, Brown and Gurdon 1964, Ritossa and Spiegelman 1965, Gall 1966c, and Brown 1966), but it has also been established that these nucleoli have their own segments of DNA, (Miller 1964b, 1966; Kezer 1965; and Lane 1967).

The first reported information about the nucleolus was by Fontana (1781). Later cytologists, for example, Carnoy and Lebrun (1897), Montgomery (1898), Marechal (1907), Jorgensen (1913), and Guyenot and Danon (1953) described nucleoli in different organisms. In amphibians, Gall (1954a) categorized the nucleoli as: (1) extra chromosomal types of nucleoli, and (2) attached chromosomal types of nucleoli. Wischnitzer (1961) described nucleoli which were vacuolated bodies of spherical or irregular shapes, between 8-15 U, and also were the smaller solid bright granules up to 5 U dia. However, Miller (1965b, 1966), Kezer (1965), Callan (1963, 1966),

MacGregor (1965), and Lane (1967) reported the existence of nucleoli in the form of rings or beaded necklaces and solid spheroid structures. Recently Callan (1966) and Lane (1967) attempted to describe how the solid spheroid peripheral nucleoli are transformed into beaded necklace rings and then back again to the solid stage. They related the change in the structure of nucleoli with the stage of oocyte maturity. Similarly, Kunz (1967) reported multiple nucleoli in Arthropods were vacuolated spheres in early stages which were later transformed to several hundred solid spheres. In the more mature stage oocytes, these spheres were either attached to the nuclear wall or connected by strings in a beaded ring form. These beaded strings were opened, stretched, and connected in a row to form long pearl types. However, there are other factors because when Miller (1964b) dispersed the nucleoli of the newt, Triturus, in diluted saline the originally spheroid nucleoli transformed into a ring shaped nucleoli.

In amphibians, Gall (1954a) and Callan (1966) established the homology of nucleoli between the mitotic and meiotic (lampbrush) chromosomes by showing that a single locus of a particular lampbrush chromosome was responsible for producing thousands of peripheral nucleoli in the oocytes, and that they were genetically related to the interphase nucleoli of the soma cell.

However, confusion still exists because all the spheres, knobs, refractile, and suspended granules which are attached along the axes of lampbrush chromosomes are known under the same name as those of similar dimension found floating free in the nucleus, i.e. nucleoli (Wischnitzer 1961; Gall 1954a, 1956; and Callan 1963, 1966). Earlier

Callan and Lloyd (1960) concluded there was no relation between nucleoli of oocytes and nucleoli of soma cells. On the basis of electron micrographs of ultrastructures of nucleoli, Wischnitzer (1961) found no homology between the nucleoli of oocytes and of somatic cells. Also Srivastava and Bhatnagar (1962) and Srivastava (1965), disagree with the single locus nucleolar organizer hypothesis. If a single locus of a particular lampbrush chromosome is responsible for the nucleologenesis then what are the other spheres found attached along the axes of the chromosomes? This question still remains unanswered.

In the chicken, soma cells have one or two nucleoli but in gametic cells there is no set pattern. During embryonic migration the primodial germ cells do not show sharply defined nucleoli (Hollander 1904, Romanoff 1960).

Using light microscopy, Hollander (1904) demonstrated that a 15 day embryonic oocyte nucleus contained a large nucleolus surrounded by a mass of chromatin. During the first 16-18 embryonic days the nucleolus was peripherally located and later along with the chromosomal parts disappeared. Approximately 6 days after hatching the nucleolus and two or three nucleoli appeared again. In an older chicken (about 20 day old) there was a large single nucleolus during lampbrush chromosome formation of oocyte nucleus.

Sonnenbrodt (1908) noted a single nucleolus in 60-80 μ oocytes of 16 day old chickens. The two or three nucleoli present during the lampbrush chromosome formation stage in the oocytes of 90-140 μ diameter later disintegrated. Brambell (1925) reported disappearance of a single nucleolus in a 10-20 μ oocyte nucleus of 4 day chick and reappearance of a single 3 μ nucleolus at the

lampbrush chromosome stage. Greenfield (1966) reported a single nucleolus in 20-100 μ oocytes of growing chicks.

The single nucleolus which Hollander (1904) observed at the early lampbrush stage later disintegrated. Concurrently several chromatic nucleoli appeared in the nuclei of more advanced stages, (Hollander 1904, Sonnenbrodt 1908, Durme 1914, and Brambell 1925). Some of these nucleoli were freely dispersed in the nucleoplasm while others were connected by chromatic threads or fragments to each other (Durme 1914).

Materials and Methods

Mature White Rock hens were obtained from the University of Alberta Poultry Farm. Nucleoli were manually isolated by opening the nuclear wall as outlined in Chapter 2. In this study Tritiated Actinomycin D, Uridine autoradiography and light and electron microscopy were used.

Results

The oocyte nucleoli observed in this study, were classified, with slight modifications, according to the classification proposed by Gall (1954a) for amphibians, namely: (I) Extrachromosomal Nucleoli and (II) Attached Chromosomal (Chromatic) Nucleoli.

I. Extrachromosomal Nucleoli

A. Giant Nucleoli

When some of the nuclei (197-366 μ dia) isolated from egg follicles (.8-2.5 mm dia) were opened in the isolation media they were found to contain free floating, discoid, rounded bodies measuring 50-171 μ in diameter (Figs. 144 and 145). These bodies will hereafter be referred to as 'giant nucleoli'. These giant nucleoli

were always single and were never observed in egg follicles larger than 3 mm in diameter. They were so bright and refractile that one could observe them in the intact nucleus during isolation and cleaning. Under phase microscope, they appeared as saucer shaped bodies composed of several refractile granules (Figs. 144 and 145).

There appeared to be no definite relation between sizes of isolated egg follicle and the nucleus and nucleolus contained in it (Table 8). For example, an egg follicle of 1.8 mm contained a nucleus of 323 μ which yielded a giant nucleolus of 171 μ dia. In another egg follicle, which was 2.5 mm and had a nucleus of 323 μ, the nucleolus was only 50 μ.

Table 8. Diameter of giant nucleoli isolated from egg follicles.

Egg Follicles (mm)	Nuclei (μ)	Giant Nucleoli (μ)
1.0	333	77.5
1.0	300	88.8
1.1	197	63.0
1.5	300	50.0
1.5	301	105.0
1.5	327	60.0
1.5	366	53.0
1.8	323	171.0
2.0	323	171.0
2.0	332	123.5
2.0	332	123.5
2.0	315	60.0
2.0	333	104.0
2.0	350	117.0
2.1	366	100.0
2.4	320	66.5
2.5	365	102.0
2.5	366	50.0
2.5	332	92.5
2.5	350	146.0

Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
1.0	2.5	197	366	50	171

Some giant nucleoli were covered with a cluster of fine radiating fibrous elements, presumably lampbrush fibers (Fig. 146). The incorporation of tritiated uridine by the giant nucleolus of Fig. 146, is shown in Fig. 147.

B. Micronucleoli

Several small bodies of heterogeneous morphology, density and refractivity were found floating free in the nucleoplasm. Collectively they are called 'micro nucleoli'. On the basis of their resemblance in morphology, density, and refractivity as well as descriptions available from the literature, they were subdivided into additional classes.

1. Solid Spheroid Micronucleoli

Variable, free-floating spheres, which because of refractivity and solid consistency appeared as scattered pearl beads of different diameters (Figs. 62, 63, 148, and 149). For example there were more than 800 in a 3 mm egg follicle nucleus and each measured about 5-8 μ in diameter. Observation under phase and plane optics of freshly isolated nuclei from egg follicles of different sizes (0.6-9 mm) indicated that although the nucleoli were varying in size and number the arrangement of the spheroid nucleoli was almost identical. In all cases micronucleoli were numerous towards the periphery of nuclei.

On opening the nucleus some of these nucleoli appeared to remain attached to the nuclear wall (Figs. 150 and 151) while others

floated free in the nucleoplasm (Figs. 63, 85, and 154). Although they were occasionally found in pairs or triplets, the conventional paired arrangement was not constant in this class of micronucleoli.

The results of tritiated actinomycin D labelling was not very encouraging (Fig. 151). However, the incorporation of tritiated uridine into the nucleoli of spheroid types indicated presence of RNA (Fig. 152).

On two occasions nucleoli from 1.25-1.5 mm egg follicles of the same bird were filled with several peculiar objects (Fig. 153). They were visible in the intact nuclei even at 20x with a stereo viewer. Upon isolation, they were observed as free floating bodies in singles, twins, or triplets and appeared as intensively refractile hollow spheres with knoblike structures (arms) radiating from their circumference. Each measured about 4-5 μ in diameter. A three hour treatment of RNase did not dissociate them. It was not seen in other birds.

The electron micrographs of isolated nuclei sections (Figs. 155 and 156) demonstrate that peripheral spheroid nucleoli have a bipartite ultrastructure with outer granular and inner darkly stained areas, which may be fibrous as was those reported by Miller (1966) on peripheral nucleoli of Triturus oocyte.

2. Beaded Ring Micronucleoli

Another class of micronucleoli appeared as large beaded rings (5 μ dia) formed by coalescence of smaller lumpy granular rings (Figs. 105, 150, 157 and 158). These micronucleoli resembled the lumpy beaded ring type nucleoli of Axolotl described by Callan (1966). Generally, the smaller beads around the rims were lumps of refractile

granular material which were uniform in diameter. After isolation these nucleoli remain attached to the nuclear envelope (Figs. 150 and 158) and when well spread, the rings enlarged and were connected to neighbouring rings by means of fine granulated strings originating from the beaded granules of the ring. These connected nucleoli gave a general appearance of a reticulum (Fig. 158).

In some nuclei only beaded rings, with the largest ring placed at one terminal, composed the axial string (Fig 157B) while in other nuclei both beaded ring types and solid spheroid type micronucleoli were attached to the same filament or axis (Figs. 157A and 157C). After fixation some nucleoli were reduced in diameter, while others disappeared. No disintegration occurred when these nucleoli were treated with trypsin and RNase. However, they were reduced in diameter in 2 hours. Small amounts of DNase dissociated the connecting string but not the rings. With large amounts of DNase the rings were dispersed (Fig. 106 and 107).

3. Pearl String Micronucleoli

Micronucleoli identified as spherical refractile pearl bead-like objects, similar to a fine long string-like necklace, were occasionally observed in the isolated nucleoplasm (Fig. 159). These micronucleoli resembled the multiple nucleoli observed in arthropods by Kunz (1967) who referred to them as beaded pearl string type of nucleoli.

The largest of these beads, ranging from 4 to 5 μ in diameter, were located at one of the terminals of the string with smaller beads, minimum 0.5 μ diameter, dispersed along the string.

In some cases the largest bead located at the terminal end of the string appeared to be floating free in the vicinity of the string. Presumably this was a mature size (about 5 μ) ready to be ejected from the main string of the nucleoli. The largest pearl bead resembled in shape, size, and refractivity the peripheral solid spheroid nucleoli discussed earlier. The only difference between the two types was that the pearl beads were attached to the string and were smaller in size than the peripheral spheroid ones.

4. Soap Bubble Type of Vesicular Structures (Micronucleoli)

Another type of peripheral object which appeared after opening nuclei of larger sizes (above 2 mm EF) was transparent vacuolated circles of vesicles of heteromorphic shape (Fig. 160). Because of their resemblance to soap bubbles or thin oil droplets, they were designated as soap bubble type vesicles (nucleoli). Variation in size and shape of these vesicles was probably due to surface tension or coalescence to others. When freshly isolated these vesicular nucleoli were rarely seen singly, rather they occurred in clusters or groups of two or more. The smaller ones were more nearly circular or spherical while the larger ones were elliptical and measured about 5-6 μ in diameter. Where their rims appeared to merge and coalesce with the neighbouring vesicles, an area of heavy granulation and refraction resulted. When they were well spread and had settled to the bottom on the glass slides they were hardly visible.

After isolation they were spread on to the nuclear membrane (Fig. 160A) where twins and triplets as well as singles measuring about 6 μ in diameter were observed. At this stage, these vesicular nucleoli appeared as perfect circles with a smooth circumference. In

all cases when freshly isolated these vesicles were concentrated towards the periphery in close association with the nuclear wall (Fig. 160B). These vesicular nucleoli were resistant to trypsin and RNase action and were not stainable with Hematoxylin.

II. Attached Chromosomal (Chromatic) Nucleoli

Although in size, shape, refractivity, general structure, and staining behaviour, chromatic nucleoli resembled the solid spheroids, the chromatic nucleoli were attached to the axes of lampbrush chromosomes rather than being peripheral and free floating. Therefore they are referred to as attached chromosomal nucleoli and were described earlier during the description of nucleolar lampbrush chromosomes (Figs. 87, 88, 89, 90, and 105). Such chromatic nucleoli were observed by earlier cytologists, (Hollander 1904, Durme 1914) who assumed them to be fragmentation products of lampbrush chromosomes in maturing oocytes of fowl.

The chromatic nucleoli were observed basically in three situations:

A. Attached nucleoli of the nucleolar lampbrush chromosomes (Figs. 67, 69, 105, and 122) were attached to recognizable chromosomal strands. These attached nucleoli were largest (4-5 μ) in the middle of chromosome axes with the smaller nucleoli located at the terminals. The basic chromosomal strand connecting them measured about 2000 Å thick. These nucleoli stained heavier than the axis when Fast green or Hematoxylin was used.

B. In large egg follicles (above 3 mm) a jumbled mass of nucleoli was observed (Figs. 89, 90, 130, 132, 133, 154, and 161). This mass

appeared similar to the 'Bizarre formation" of oocyte nucleoli described in earlier works (Montgomery 1898; Jorgenson 1913). However, these chromatic nucleoli always showed a definite structural pattern and ranged from very minute to 5 μ in diameter. They were solid, spherical, refractile and were always attached to fragmented or ribbonlike strands of chromosomal elements. Using Fast green and Hematoxylin (Figs. 90 and 161) the nucleoli stained similar to the attaching strands. However, response to Feulgen reaction was poor for both nucleoli and attaching strands.

When the same source material was placed on grids (whole mount preparation) and observed under an electron microscope, both the axial strands and attached nucleoli appeared electron dense and had been deeply stained by Uranylacetate (Figs. 130, 132, 133, and 154). The nucleoli were attached by means of helically coiled, bipartite strands which may have been loop material from chromomeres of the lampbrush chromosomes. These strands were ribbon-like or beaded filaments which demonstrated periodic constrictions and expansions along the axis. The loop materials within which these nucleoli were contained consisted of strands of two different thicknesses (Figs. 130 and 133). The finer strands measured about 150-200 Å and merged into the thicker strands. It appears that the finer strands were the uncoiled strands of the main loop.

A similar situation was observed in the inter-bead regions of the pearl string type nucleoli which were submicroscopic and approximately 250 Å thick. At places, two of such 200-250 Å strands could be deciphered as forming nucleoli (Figs. 130 and 132). Beads of these strands enlarged more than the axis and appeared as spheres of the so-

called nucleoli. The smallest size of nucleoli (granules) which was attached with the thicker and denser strands had a diameter of about 600 Å while with the finer strand the smallest attached granules (nucleoli) were about 300 Å. The biggest nucleolus in the preparation was about 2.5 μ (Fig. 133). A large nucleolus in the same preparation was involved in budding to smaller granules from its circumference (Fig. 132).

C. Some nucleoli were similar in dimension, density and morphology to those described above except all of the attached nucleoli appeared to be of the same radius dimension and were attached to branched, slender chromosomal elements (Figs. 87 and 88).

Discussion

In the absence of information on the morphology of nucleoli isolated from chicken oocyte nuclei, comparison of the results obtained in this study can only be made with other species.

Presence of a single or double nucleolus is a common occurrence in somatic cells of animals and plants whose karyotype contains a corresponding nucleolar chromosome organisor, (Frankhauser and Humphrey 1963). Large nucleoli have been observed in starfish oocyte (Fieq 1955 and Bal et al. 1969), in cat oocyte (Morato 1966) and in some insects (Bier et al. 1967). However, large size nucleoli (50-170 μ dia) as reported for the giant nucleoli in this study appears to have never been reported in somatic or oocyte nuclei of animals. Some light microscopists (Hollander 1904, Durme 1914, Brambell 1925, and Sluiter 1939) and an electron microscopist (Greenfield 1966) have reported the presence of large

(3-5 μ) single nucleolus in the nuclei of growing fowl oocytes.

Results obtained earlier in this study on stained sections prepared from growing and mature bird oocytes (Chapter II) have indicated the presence on the nucleus of a large nucleolus contemporary with lampbrush chromosomes. This is similar to the findings of Hollander (1904) and Sonnenbrodt (1908). Although with smaller oocytes there were smaller nucleoli, the presence of a single giant nucleolus in mature bird oocytes (EF up to 2.5 mm) and the contemporary presence of lampbrush chromosomes raises the question of how two oocytes of different sizes and ages can maintain a similar state of nucleoplasm in which the nuclear apparatus bearing lampbrush chromosomes and a large nucleolus, are present. It could be that such a state may be characteristic of a common event of the nuclear metabolism such as storage of RNA ready to be used for active synthetic purposes for the oocytes at different intervals of their life cycle. In fowls this may be in 18 day chicks or mature birds, in either small or large oocytes.

The intensity of uridine incorporation was maximum in the body of the giant nucleolus. The fibers attached to the nucleolus indicated a gradient flow of labelling from the body of the nucleolus to the distal ends of the fibers. Were these fibers from a single chromosome, or a single nucleolar organizer? It would be premature at this time to establish a homology between this giant nucleolus with the somatic nucleolus, however, Ohno *et al.* (1962) counted twelve or more microchromosomes which they proposed acted as nucleolar organizers in the mitotic cells of the fowl.

It is conceivable that these fibers covering the nucleolus

may be the lampbrush stage of microchromosomes and are involved in nucleolar organization. Evan's (1967) recent report of human oocyte nucleolus and its attachment to the acrocentric chromosomes is similar to the result of this study. Nevertheless, persistence of a nucleolus during the entire dictyate phase of human oocytes as referred by Evans (1967), do not agree with the present finding of the fowl oocytes. Whatever may be the case, incorporation of uridine in the body of the giant nucleolus and containment of RNA by the attached radiating fibers fulfills the basic requirements for a structure to be known as nucleolus (Perry 1966, 1967).

In fowls, the 18 day chick oocyte contains a single large nucleolus at the lampbrush stage. In mature hens, not only a giant nucleolus but also several peripheral nucleoli were observed concurrently with lampbrush chromosomes. Later (above 2.5 mm EF) the large nucleolus disappeared leaving lampbrush chromosomes along with several attached nucleoli and hundreds of extra chromosomal nucleoli (micronucleoli) which existed in increasing numbers as the development of the oocyte advanced. On the basis of the information obtained in this study these peripheral nucleoli; described as Solid spheroid, Beaded ring, Pearl string types micronucleoli; must have been produced simultaneously with the lampbrush phase of chromosomes.

Micronucleoli with more or less similar configurations have been described in amphibians (Guyenot and Danon 1953; Gall 1954a; Wischnitzer 1961; Callan 1966; Lane 1967; and Miller 1964b, 1966), insects (Kunz 1967; Bier et al. 1967), starfish (Vincent 1964), and in other animals. The appearance of a multi-nucleolar condition in amphibian oocytes has been suggested by MacGregor (1965) as governed

by two general mechanisms. The first mechanism based upon the observations on amphibians by Gersch (1940), Guyenot and Danon (1953), Miller (1962b) and MacGregor (1965), and on fishes by Chouinard (1963) is that the peripheral nucleoli were formed before or soon after the chromosome had assumed the lampbrush form. However, MacGregor considered there were two exceptions to the first mechanism, namely two species of Triturus e.g., T.C. Cristatus and P. Cinerous and that these followed the second mechanism. This second mechanism had previously been outlined by Duryee (1941, 1950), Gall (1954a), and Srivastava and Bhatnagar (1962). These authors believed that the peripheral nucleoli of amphibian oocytes were produced or shed by one (Gall 1954a) or more loci (Srivastava 1965) on the lampbrush chromosomes, and these organizers were characterized by attached spherical objects which were similar to free nucleoli.

It is well established that growing oocytes of animals contain multiple nucleoli (MacGregor 1965, Lane 1967). The transformation in the chemical composition of peripheral nucleoli associated with changes in shapes, sizes and outlines of the oocyte nucleoli (Miller 1966, Bernhard 1966, and Lane 1967) may be a reflection of a series of metabolic changes in the nucleoplasm which must be coupled with the extraordinary high rate of RNA synthetic process of growth (Brown and Ris 1959, Brown 1966; and Miller and Beatty 1969).

However, the structural changes of the oocyte nucleoli are challenged by Miller (1964b, 1966) who maintained that the observed changes in the shape and structure of the nucleoli were due to

differences in tonicity of the isolation media (diluted saline). Miller and Beatty (1969) recorded changes in ultrastructure of peripheral nucleoli isolated in media of different ionic strengths. The situation is complicated by the observations made by Kezer (1965), who consistently found that the nucleoli of Salamander oocytes had sizeable rings.

During isolation studies beaded ring and solid spheroid micronucleoli were the most common form of the nucleoli in the nucleoplasm. Therefore, at this time, it is difficult to state specifically whether these structurally different nucleoli (solid spheroid, beaded ring, pearl string, and vesicular type) occur normally in nuclei of the growing oocytes of fowl or they represent the transitional forms.

The other types of nucleoli such as the attached chromosomal, the beaded ring, the pearl string types, very closely resembled the solid spheroids in shape, size, stainability, and refractivity. Furthermore, the solid spheroid and the beaded ring type nucleoli were observed in close vicinity to each other on the same piece of nuclear membrane and even on the same fiber. Therefore the beaded ring type may be related to or transformed to or from the spheroid type. It is doubtful that the sequence for the transitional scheme from the solid spheroid to beaded ring and back as has been proposed in amphibians by Callan (1966) and Lane (1967) is as simple in the fowl.

Soap bubble type of vesicles were the most interesting objects of the oocyte nucleus observed in this study. They appeared as large vacuoles which may have been formed by coalescence of several

small vacuoles such as has been commonly described for the vacuolated type of the peripheral nucleoli of amphibians (Gall 1954a, MacGregor 1965, and Callan 1966) and of lung fish (Scharrer and Wurzelmann 1967). On this assumption, plus the fact that a vacuolated type of peripheral nucleoli was never observed in the isolation study, these vesicles may be regarded as vesicular micronucleoli, and if so this may be a species variation.

Electron micrographs prepared from 1-2 mm egg follicles resolved very minute vesicular objects (microvesicles) inside the nuclei and close to the envelope. However, vesicles of similar dimension and larger were also observed close outside the envelope in the cytoplasm. As these vesicles observed in electon micrographs were very minute as compared to those observed after isolating the same size of egg follicle and opening the nucleus (phase microscopy), an attempt to relate the two can be made only on the assumption of shrinkage under fixation and embedding of the specimen.

The possibility exists that these vesiculated nucleoli which were attached to the inner side of the isolated nuclear membrane may be related to the annulate lamellae of nuclear origin (Merriam 1959; Hsu 1963; Kessels 1964; 1965; and Folliot 1968). Although there are several hypotheses regarding the origin of the cytoplasmic annulate lamellae, observations of Wischnitzer (1963) regarding the origin and formation of microvesicles from the oocyte nuclear wall appears related to the results obtained in this study.

Based upon electron microscopic observations of vesicular structures associated with the nuclear wall, both inside and outside the oocyte nucleus, one may conceive the passage of these structures

across the nuclear wall. Reports have appeared suggesting that nucleoli can move bodily from the nuclear membrane to the cytoplasm (Choudhary 1951, Wittek 1952, Dodson 1953, and Srivastava 1965). In the fowl, an indication of movement of nucleoli to the cytoplasm during the late growth period was reported as early as 1906 by Loyez and 1908 by Sonnenbrodt. In other animals, strands of granular material extending from the region of the nucleolus to the region of oocyte cytoplasm have been observed by Wischnitzer (1958) and Beams and Kessel (1963). Beams (1964) even suggested this as the probable means of passage of macromolecules from the oocyte nucleus to the cytoplasm in crayfish oocyte. Passage of ribosomal elements across the nuclear wall has been reported by Szollosi (1965) in rats, Stevens and Swift (1966) and Steven (1966) in amoeba, Scharrer and Wurzelmann (1967) in lungfish, Anderson (1956) in pipefish, and Elizabeth (1968) in amphibians. However, Callan (1966), MacGregor (1965), and Gall (1954a) do not support the idea of bodily movement of nucleoli from nucleus to cytoplasm. They believed a fault in sectioning may have caused nucleoli or nucleolar material to be observed in the cytoplasm.

In a few specimens of nuclei which were isolated, fixed, and embedded under stereo observation, the electron micrographs demonstrated a dragging-over effect of ribosome-like elements from the peripheral nucleoli, either due to faulty sectioning or the dragging effect of razor. Although this may agree with Gall's (1954a) conclusion in a number of micrographs these vesicular objects were not only located closer to the nuclear wall on either side but

within the reticulum of the nucleoplasm. To solve the question of bodily movement of nucleoli or the nucleolar material to the cytoplasm, an additional experiment using labelled material specific to nucleoli is necessary.

Another point of interest is whether there is a single nucleolar organizer locus of a lampbrush chromosome (Gall 1954a, Callan 1966) or several (Srivastava and Bhatnagar, 1962) responsible for the nucleologenesis. A single nucleolar organizer is conceivable for the production of a few nucleoli, as in somatic cells, but the production of hundreds of nucleoli associated with a single locus in a lampbrush chromosome would require a continuous active synthesis of nucleoli. If this were true then the production of the nucleoli from the nucleolar organizer could be demonstrated with movie microphotographs. Alternatively Srivastava and Bhatnagar (1962) and Srivastava (1965) suggested that the nucleolar material arises from the entire chromosomal surface of the chromosomes and the nucleolar organizer only represents the area of accummulation of the material synthesized. On the basis of the large spheres and rounded refractile bodies which are found associated with the long axes of lampbrush chromosomes and their lateral loops, it seems most likely that the production of such a large number of nucleoli may be from several sites rather than one site.

The overall picture of oocyte nucleoli suggests that the lampbrush chromosome loops radiate from the chromomeres. Either the main strands of lampbrush chromosomes or their loop axes develop the spheroid and beaded ring nucleoli and these are shed individually after reaching a required size; or the lumpy loop of

chromosomes bearing several spheroid nucleoli became uncoiled and form pearl string type of nucleoli which after maturity leave the string and became peripheral spheroid nucleoli.

Fig. 144. A 307 μ nucleus from a 1.5 mm EF isolated and flattened on slide showing 105.7 μ giant nucleolus (NL) and the intact nuclear wall (W). Phase. Magnification approximately 350 X.

Fig. 145. A 350 μ isolated nucleus from a 2 mm EF showing 117 μ giant nucleolus (NL) and nuclear wall (W). Phase. Magnification approximately 100 X.

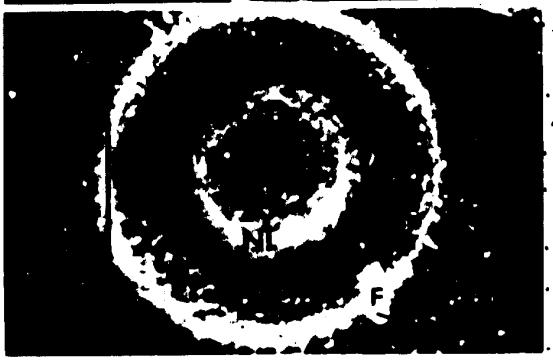
Figs. 146, 147. A 116 μ giant nucleolus (NL) isolated from 366 μ nucleus, 2.2 mm EF showing attached lampbrush chromosome fibers (F). Fig. 146. Prior to autoradiography; Fig. 147. After uridine autoradiography. Phase. Magnification approximately 400 X.

Fig. 148. A 350 μ isolated nucleus from a 2 mm EF showing fuzzy appearance of nucleoplasm. Phase. Magnification approximately 400 X.

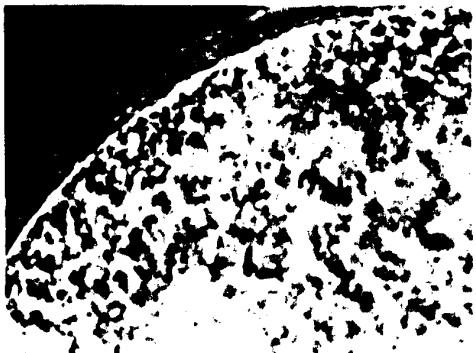
Fig. 149. A 336 μ nucleus from a 3 mm EF showing solid spheroid nucleoli (S) towards periphery. Phase. Magnification approximately 400 X.

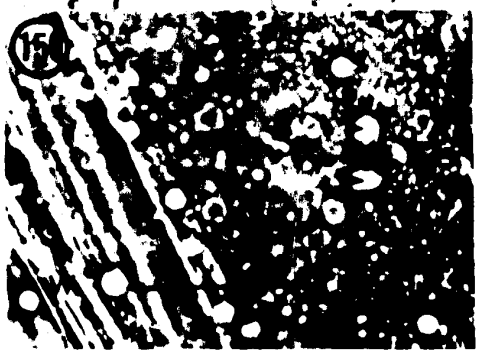
Figs. 150, 151. Spread envelope (W) of 250 μ isolated nucleus from a 2.5 mm EF showing solid spheroid nucleoli (S). Fig. 150. Before autoradiography showing beaded ring type nucleoli (B); Fig. 151. After actinomycin DH^3 autoradiography. Phase. Magnification approximately 1,150 X.


144


Nt
F


147
Nt
F




15


149
N
S


151
S

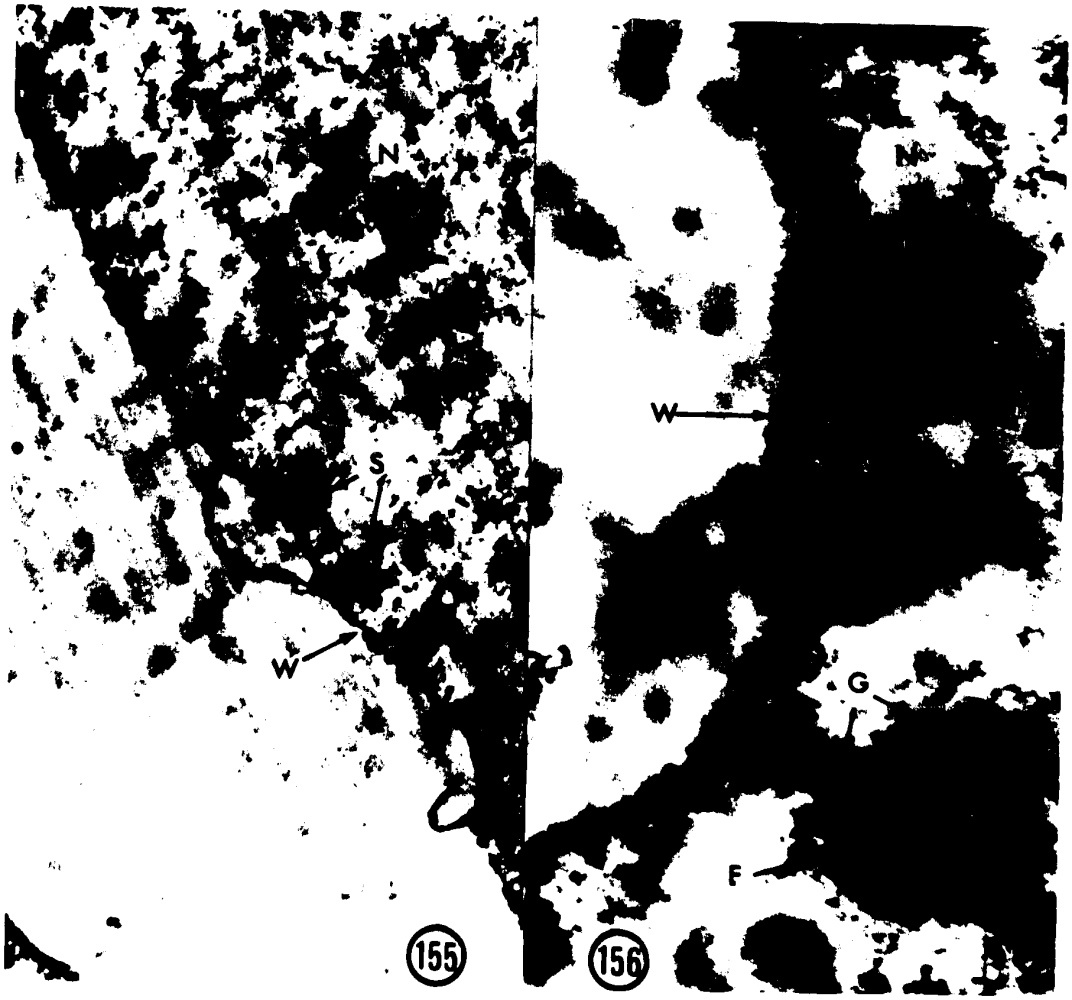
Fig. 152. Solid spheroidal micronucleoli (S) isolated from 6 mm EF, showing tritiated uridine incorporation. Phase. Magnification approximately 1,200 X.

Fig. 153. Peculiar nuclear objects with side arms (A) isolated from 350 μ nucleus 1.5 mm EF. Inset contains sketch of the object. Phase. Magnification approximately 1,000 X.

Fig. 154. A 400 μ nucleus; 4 mm EF, isolated and exposed on grid showing chromatic nucleoli (CG). EM 100. Magnification approximately 9,000 X.

Figs. 155, 156. Isolated nucleus; 0.1 μ thick section, showing spheroid nucleoli associated with nuclear wall (W). Fig. 155. Peripherally located spheroid nucleoli (S). EM 100. Magnification approximately 15,600 X. Fig. 156. Nucleolus showing outer granular (G) and inner darkly stained area (F). EM 100. Magnification approximately 45,000 X.

154



155 156

Fig. 157. Beaded ring nucleoli (B) isolated from 300 μ nucleus; 1.5 mm EF, joined in twins or triplets. Attached to same axis to which chromatic nucleoli (CG) attached. Phase. Magnification approximately 1,000 X.

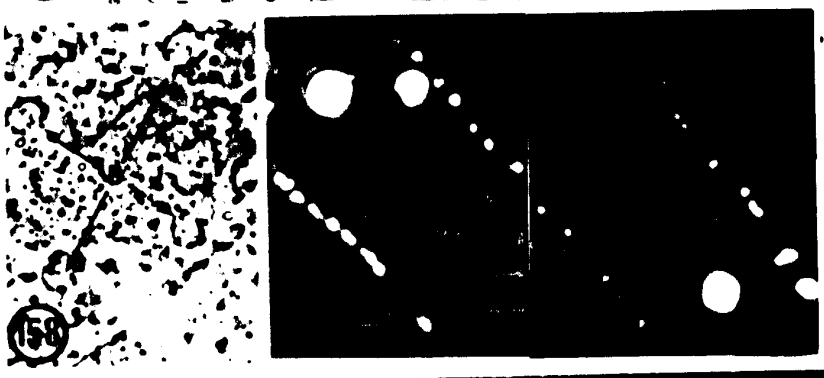
Fig. 158. Beaded ring nucleoli (B) isolated from 300 μ nucleus; 2 mm EF. Phase. Magnification approximately 1,000 X.

Fig. 159. Pearl string micronucleoli (P) isolated from 400 μ nucleus; 4 mm EF. The larger granules (CG). in Fig. 159 A, ejected away from the array, while smaller attached to the string in Fig. 159 B. Phase Magnification approximately 1,000 X.

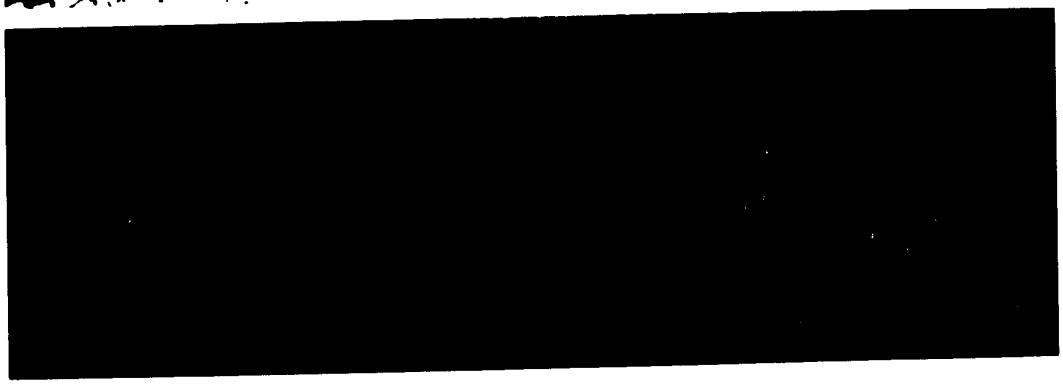
Fig. 160. Soap bubble type vesicles (SV). Fig. 160 A. Spread onto the nuclear wall (W). Magnification approximately 1,000 X. Fig. 160 B. Immediately after opening the nucleus the vesicles (SV) remained attached to the brim (R) of the torn nuclear wall (W). Phase. Magnification approximately 3,400 X.

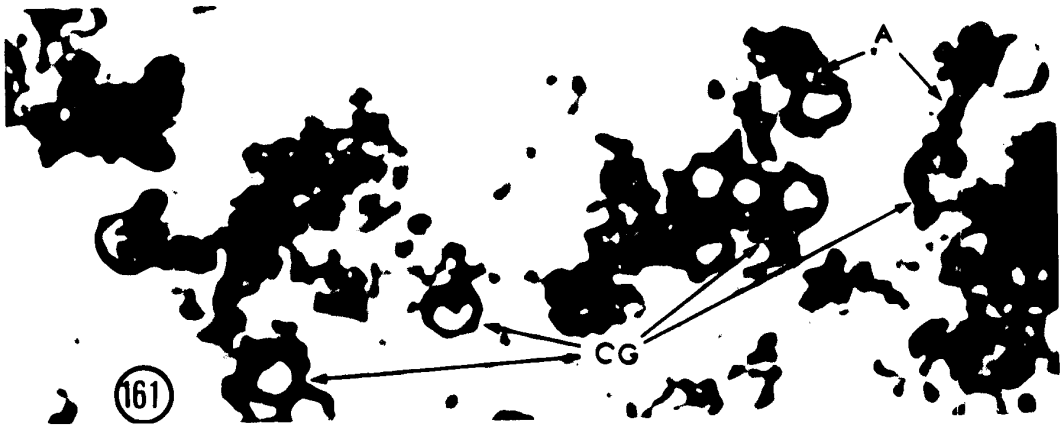
Fig. 161. Chromatic nucleoli (CG) attached to chromatic axis (A), isolated from 366 μ nucleus; 3 mm EF. Hematoxylin stained. Magnification approximately 300 X.


157
A


158


V
SV
160
A




A
CG
161

NUCLEAR ENVELOPE

Literature Review

The presence of a wall delimiting nuclear content from other parts of the cell was suspected as early as 1883 by Brown. In 1896, Flemming proposed that the wall was double. Although in retrospect, the micromanipulation experiment of Chamber and Fell (1931), and the birefringent data of Schmidt (1939) and Monne (1942) were sufficient to reveal the bilamellar nature of the nuclear envelope, it was not until after information reported in the late 40's and early 50's that a clarification of the main structure of the nuclear envelope was obtained.

Even though early cytogeneticists were using oocyte nuclei of amphibians (Brachet 1929, 1940; Duryee 1937, 1940, 1941; Waddington 1938; and Gersch 1940), chickens (Hollander 1904, Sonnenbrodt 1908, Durme 1914, and Koltzoff 1938) and other animals (Revene 1961), no special attention was paid to the envelope structure until 1950, when Callan and Tomlin produced electron micrographs which had been prepared by shadowing the surface of the nuclear envelopes isolated from triturus and xenopus oocytes. Although their finding that the nuclear envelope was composed of two layers, an outer porous layer and an inner continuous layer, was controversial, their technique was widely used in other studies, e.g., (Bovey 1952; Gall 1954b, 1959, 1967; Merriam 1961a, 1961b, 1962; and Frank 1966). Because the two membranes collapse when the isolated envelope is mounted on the grid (Gall 1964), information from the study of sectioned materials (Hartman 1953; Pollister et al. 1954; Afzelius 1955; and Watson 1955, 1959) is valuable for a clear illustration

of bilamellar nature of the envelope.

The presence of a bilamellar nuclear envelope with pores containing annuli, as indicated by Frank (1966), has been reported in all oocytes which have been studied (Norrevang 1968). The major reports are of Callan and Tomlin (1950), Gall (1954b, 1959, 1964, 1967), Pollister et al. (1954), Afzelius (1955), Rebhum (1956), Swift (1956), Wischnitzer (1958), Barnes and Davis (1959), Merriam (1962), Odor (1960), Sichel (1966), Frank (1966), Norrevang (1968), and Kessel (1965).

On the basis of these studies, a general structure of the nuclear envelope may be described. The nuclear envelope consists of two parallel identical membranes similar to the cytoplasmic membrane (Gall 1964). Each membrane varies from a minimum of 75 Å to a maximum of 100 Å in thickness (Gall 1964). The two membranes are separated by an interlamellar space, called 'perinuclear space', of about 100 - 300 Å (Watson 1955). Each single membrane of the nuclear envelope is tripartite with each part measuring about 30 Å (Gall 1964).

In transverse sections, the nuclear pores appear as a discontinuity in both membranes of the nuclear envelope at the edges of which the inner and outer membranes are continuous, or they fuse in an area approximately 500 Å in diameter (Norrevang 1968). The pore diameter ranges from a low of 300 Å (Afzelius 1955) to a high of 1000 Å (Watson 1959).

Tangential sections of the nuclear envelope show dense rings, similar in dimension, called annuli. Although Callan and Tomlin (1950) were the first to observe annuli, Afzelius (1955) was the first to propose the material to be associated with, rather than an integral part, of the pores. The literature on annuli contains a wide variation in the reported dimensions as well as in their proposed structure

(Afzelius 1955, Watson 1955, Wischnitzer 1958, Gall 1959, and Merriam 1962).

Although detailed information of the chicken oocyte nuclear envelope is not available, Franceschini and Santro (1965) recently reported the presence of nucleopores in the germinal cell of a nine-day old chick embryo, and Greenfield (1966) observed 600 - 800 Å diameter nucleopores in day old chick oocytes.

Because of this lack of information on the chicken oocyte nuclear envelope in the fowl, a study of the structure of the nuclear envelope was made.

Materials and Methods

Ovarian tissue was removed from White Rock hens obtained from the University of Alberta Poultry Farm.

Three approaches to the study of the nuclear envelope were made. The first was light microscope studies of stained sections of ovarian tissue. The second was the electron microscope studies of stained sections obtained from isolated nuclei, from isolated egg follicles of mature birds, and from the ovarian tissue of a seven week old chick. The third was a study under the electron microscope of the surface view of the nuclear membrane preparations which were spread by tearing the envelope of the isolated nucleus in (5:1) isolation media. During the isolation, the membrane was observed under a stereoscope and the grid placed below the membrane. The grid was later lifted with the nuclear membrane on it.

Results

As early as 1904 Hollander, in his classical cytological study, demonstrated the presence of a double walled envelope around the nuclei of chicken oocytes. This was later confirmed by Loyez (1906), Sonnenbrodt (1908), and Durme (1914) who explained the envelope as a corrugated undulating double wall around the growing oocyte nuclei. They considered the outer wall of the nuclear envelope as a condensation product of the cytoplasm and that this outer wall formed the inner cytoplasmic membrane. Observation of the stained sections of ovarian tissue from the growing chick (Figs. 30A and 31) suggests that the two walls have been pulled apart, possibly due to an effect of the fixative, dehydration, or from dragging of the microtome blade. Figures 30A and 31 show how this could have been a confusing factor for the early light microscopists.

In sections of isolated nuclei, isolated egg follicles of hens, and a seven week old ovarian oocyte, a bilamellar organization of the nuclear envelope was resolved (Figs. 135, 137, 162 and 162A). Each membrane of the envelope was approximately 100 - 125 Å thick and the interlamellar space, prenuclear space of Watson (1955), was approximately 175 - 200 Å wide. Therefore the complete nuclear wall was approximately 350 - 450 Å thick.

Although in cross sections the nuclear pores, about 600 - 800 Å in diameter, could normally be observed (Fig. 162A), a sharp boundary of the nuclear pore was difficult to resolve because of the electron dense material of annuli which sometdmes masked the pore boundaries (Fig. 162).

In oblique sections (Fig. 142), the annuli measuring 1000 Å

(outer dia) appeared as spherical rings of electron dense material arranged in two or three complete or incomplete rows. However, in tangential sections the annular rings appeared in a single file around the circumference of the nucleus (Figs. 143, 163, and 163A). The annuli of two adjacent rows appeared in a triangular array. The distance between two adjacent annular rings of the same row was about 1000 - 2000 Å, while the distance between two annuli of two adjacent rows was about 500 - 1000 Å.

Surface views of the spread nuclear envelope, phase (Fig. 164) and electron microscope (Fig. 165), did not provide sufficient information to clarify the structure and nature of annular rings or of the pores. Although under phase observation the surface view suggested a pitted nature of the nuclear envelope, a careful examination indicated that this pitted appearance was more likely due to peripheral granules of the nucleoplasm which remained attached to the transparent nuclear envelope (Fig. 164).

Vesicular structures (microvesicles) were generally observed in the nucleoplasm (Figs. 135, 136, 137, 138, and 139). From observation of these micrographs one may consider these microvesicles to be involved with the nucleocytoplasmic transport system as they appear to pass across the nuclear wall (Fig. 166). Formation and nature of these microvesicles was discussed earlier (Chapter III) and are considered to be related to the multiple vesicles of the cytoplasm. However, in a few sections which appear to be sagittal, the microvesicles appear to be empty annular rings (Figs. 134 and 167).

Although in thick sections of isolated nuclei, blebbing or roughness of the outer membrane was more evident, a bilamellar nature of

the envelope was quite distinct. However, the discontinuity of the envelope (nuclear pores) at places appeared to contain a partition-like connection (diaphragm) instead of a clear discontinuity in the envelope (Fig. 168).

High magnification of thin sections viewed under electron microscope showed the outer membrane of the nucleus to be involved in blebbing and forming folds and corrugations, while the inner nuclear membrane remained smooth (Figs. 162 and 169).

Although the annuli in surface view showed heavier stain than the background material (Figs. 163, 163A, 170, 171, and 172), in the center of the rings a few annuli were either faint or clear. Some of the annular rings in the surface view appeared to contain about 6 granular or vesicular materials (Figs. 162, 171, 172). It is assumed that the annuli of amphibians contained 8 - 10 lumps of granules or spherical masses, Gall (1954b, 1956) or subannuli (Rebhun 1956).

Extra evidence of microvesicles being involved in nucleocytoplasmic transport system (Fig. 166) may be obtained on the basis of existence of such microvesicles and/or the granular substances found in the rings of pores, and prenuclear space, and immediately in and outside the nuclear wall (Figs. 163A, 169, 170, 171, 172, and 173).

In some of the sections containing surface views of the annuli (Figs. 140, 163, and 173) the center of the annular rings contained a fine dark spot which appears to be 'central granules' of the annulus.

Discussion

There is no doubt that the nuclear envelope in the fowl oocytes is bilamellar in nature. However, the membrane thickness of the nuclear wall obtained in this study, 100 - 135 Å, was slightly higher than the value of 75 Å reported by Wischnitzer (1958) and closer to the thickness of 75 - 100 Å reported by Gall (1964).

At intervals the two membranes appear to be united to each other, thereby causing discontinuity (nucleopores) in the nuclear envelope. The nucleopores were associated with the annular material. Although the electron micrograph prepared from a thick section of an isolated nucleus resolved a partition-like structure between the discontinuities (pores), a distinct diaphragm resulting from fusion of the two membranes as observed by Afzelius (1955) was not observed in any thin section. Presence of such a diaphragm has been denied by several investigators (Luft 1956; Barnes and Davis 1959; Wischnitzer 1958; Gall 1964, 1967; Kessel 1965; and Sichel 1966).

The thickness of the inner and outer nuclear membrane and the interlamellar space between the two unit membranes of the envelope in specimens of 7 week old chicks were similar to those of laying bird oocytes. This lack of variation suggests that growth of the oocyte corresponded with growth of the nuclear membrane, and also isolation of the nucleus in the 0.1 M (5:1) solution caused no expansion of either the perinuclear space or of the two unit membranes of the envelope. This would be in disagreement with the mechanisms on 'nucleocytoplasmic exchanges' through prenuclear space of the envelope outlined by Watson (1955).

The diameter of nuclear pores of chicken oocytes as reported in this study is in agreement with Wischnitzer (1958), Gall (1964b, 1959), and Greenfield (1966). The density of the pores in this study was about 12 to 18 per sq. mic. Using the procedure of Gall (1964) the data obtained in this experiment indicates that 15% of the nuclear envelope of the fowl oocyte would be covered by pores. This value falls between the values of Watson (1955) and of Merriam (1962).

Surface view of the nuclear envelope by mere transmission electron microscopy could not contribute much information in this study because the lack of shadowing and proper dehydration (Anderson 1951). The relationship between the two membranes of the isolated envelope may have changed during mounting on the grid. Therefore to determine whether the nuclear pores in fowl oocytes are octagonal or not (Gall 1967) needs further studies.

The diameter of the pores appears to be smaller than the outer diameter of the annuli. This does not agree with Afzelius (1955), Watson (1959), and Wischnitzer (1958), but agrees with Gall (1954b, 1959) and Merriam (1961a), who reported the pore diameter in amphibians was less than the outer diameter but greater than the inner diameter of the annulus.

In cross sections showing the discontinuity of the nuclear envelope (nucleopores) the electron dense materials (vertical section of the annuli) projected both above and below the level of the nuclear membrane. Similar observations led Afzelius (1955) to visualize the starfish annulus as a cylindrical structure, extending 600 Å into the nucleus and 150 - 200 Å into the cytoplasm; and Wischnitzer (1958) to propose the annulus consisting of a group of cylinders.

However, in section studies, viewed through the electron microscope, of the surface view and vertical aspect, the annuli were easily resolved to contain annular granules or vesicular structures. Similar results were obtained by Gall (1954b, 1964), Rebhun (1956), Sichel (1966), and Norrevang (1968).

As in this study, investigators in other studies have reported the presence of electron dense material in the centre of annuli (Gall 1954b, Pollister et al. 1954, Afzelius 1955, Swift 1956, Gay 1956, Wischnitzer 1958, and Watson 1959). This electron dense material has been termed 'central granule'. Merrium (1962) observed in amphibians the central granules occurring more frequently in early vitellogenesis than in late stages. Interestingly enough, central granules have been implicated as a source of binding material in the process of nucleocytoplasmic exchange of materials by Feldherr and Harding (1964).

Fig. 162. Nucleocytoplasmic region from 7 week fowl oocyte showing inner (IW) and outer (OW) nuclear membrane, the perinuclear space (PN), nuclear pores (P), the annular dense material (AM), and Blebbs (B) of outer membrane. The cytoplasm (CY) contains ribosome-like particles (R). EM 100. Magnification approximately 75,000 X; Inset A. Part of Fig. 162. showing nuclear envelope (n) and clear nuclear pore (P). EM 200. Magnification approximately 14,000 X.

Fig. 163. Tangential section of nucleocytoplasmic region from 7 week fowl oocyte showing annular rings (A) containing central granules (G), granular or vesicular elements (AV) and cytoplasm (CY) containing many mitochondria (M). EM 100. Magnification approximately 55,000 X. Inset A. Nuclear microvesicles passing to cytoplasm through nuclear pore region. EM 200. Magnification approximately 15,000 X.


AM
P
N
PN
OW
IW
CY
N
R
B
AV
G
AV
N
CY
AV
MV
A
162
163

Fig. 164. Surface view of nuclear wall isolated and spread on slide showing nuclear granules (CG). Corner turned over. Magnification approximately 1,200 X.

Fig. 165. Surface view of nuclear wall isolated and spread on electron microscope grid. EM 100. Magnification approximately 9,000 X.

Fig. 166. Nucleocytoplasmic region, 0.1 μ thick section of isolated EF showing nuclear microvesicles (MV) crossing the nuclear membrane (arrow). EM 200. Magnification approximately 25,000 X.

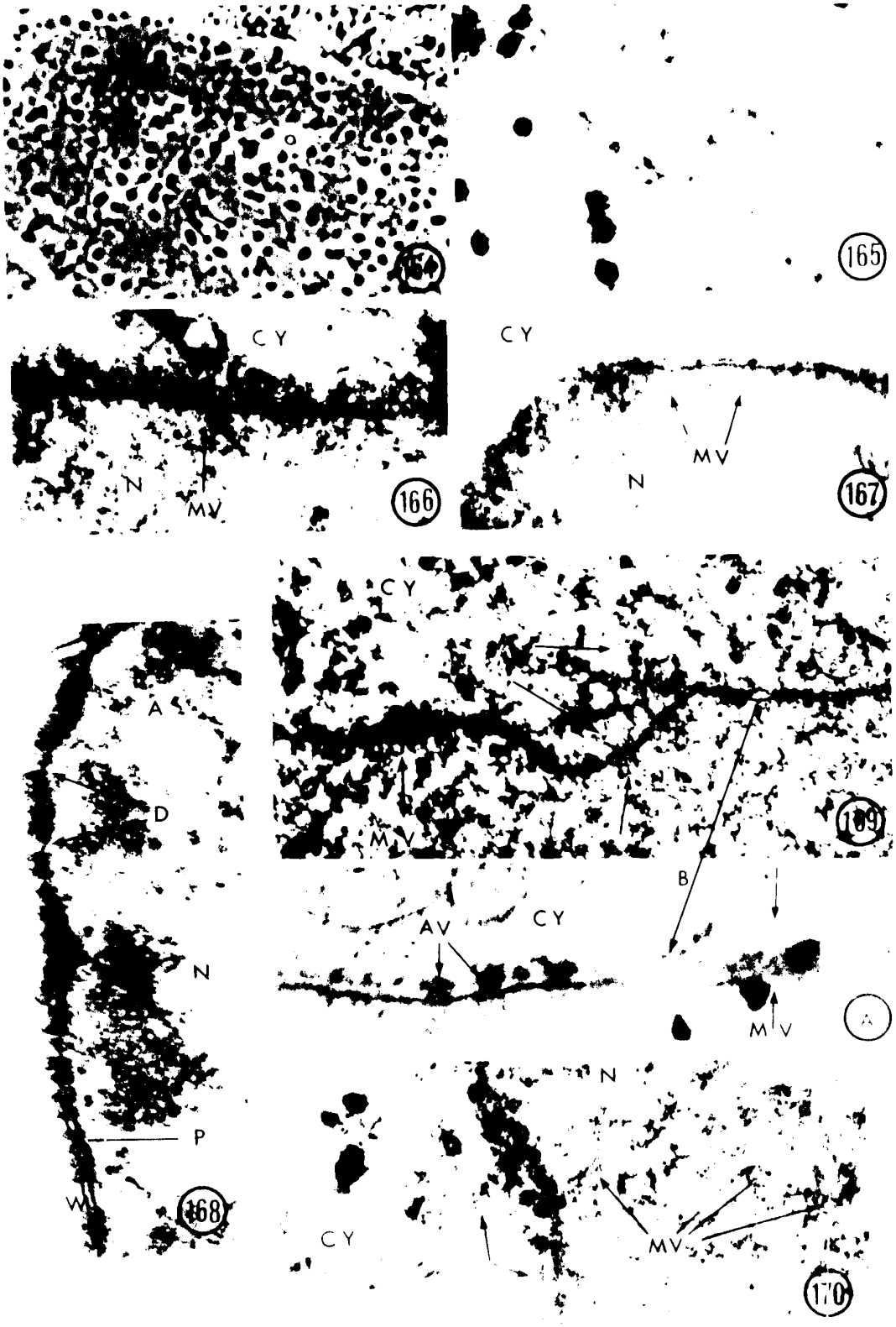
Fig. 167. Section (.1 μ thick) of isolated nucleus showing microvesicles (MV). EM 100. Magnification approximately 21,600 X.

Fig. 168. Section (.1 μ thick) of isolated nucleus (2 mm EF). Arrows from D indicate nucleopores bridged by a diaphram-like partition (D), nucleopores (P) and annular rings (A). EM 100. Magnification approximately 51,000 X.

Fig. 169. Nucleocytoplasmic region of 2 mm isolated EF, showing reticulated nucleus containing microvesicles (MV) identical to cytoplasmic microvesicles (arrows), blebbing of outer nuclear membrane (B); EM 100.

Magnification approximately 13,000 X. Inset A. The nuclear membrane (B) showing outer nuclear membrane forms blebbing and small arrows indicate annular vesicles (AV). EM 100. Magnification approximately 52,000 X.

Fig. 170. Nucleocytoplasmic region of 7 week fowl oocyte showing presence of microvesicles (MV) in the nucleoplasmic reticulum. Unlabelled arrows indicate identical vesicles in cytoplasm (CY). EM 100. Magnification approximately 22,000 X.


164
165
CY
CY
MV
N
166
MV
N
167
CY
A
D
N
P
168
MV
B
AV
CY
169
A
MV
N
CY
MV
170

Figs. 171, 172, 173. Nucleocytoplasmic region (600 Å section) of 7 week old fowl oocyte. Fig. 171. Shows projected annular material (AM) both inside and outside the nucleus (N). The annular rings in cross section contains about 6 dense granules (AV). The cytoplasmic vesicles are in close association with outer nuclear membrane (arrow). EM 100. Magnification approximately 52,500 X. Fig. 172. Shows annular granulations (AV) are clear on a surface view of sectioned annulus. Arrow indicates very close association of cytoplasmic vesicle with nuclear wall. EM 100. Magnification approximately 42,000 X. Fig. 173. Shows nuclear microvesicles (MV) more evident, one annulus shows central granule (G). Arrow indicates cytoplasmic vesicles appear like annulate lamellae. EM 100. Magnification approximately 42,000 X.


CY
AV
N
AM
171
MV
CY
N
N
CY
G
AV
172
173

OSMOTIC BEHAVIOUR OF OOCYTE NUCLEI

Literature Review

Osmotic studies on isolated or intact nuclei have been a vital area in biological research for studies on nucleocytoplasmic relationships, isolation of cell organelles, and tissue culture. Although the importance of knowledge of the permeability of the isolated nucleus in modern biology has been recognized (Mitchison 1966), the osmotic behaviour of the fowl oocyte nuclei has not been established.

The earliest study of the osmotic properties of the nucleus was made by Hamburger (1902). In studies by Beck and Shapiro (1936), Shinke (1937), Buck and Boche (1938), and Churney (1942), the nucleus swelled when placed in salt solutions of different concentrations which indicated an osmotic behaviour of the nucleus. This swelling was due to the passage of the electrolytes across the nuclear membrane.

Intact oocytes which were treated with radioactive sodium (Abelson and Duryee 1949) demonstrated not only the rapid transfer of ions into the nucleus but also their accummulation when KCL and NaCl solutions were injected into the ground cytoplasm (Harding and Feldherr 1959). Moreover Naora et al. (1962), using radioactive tracers, observed not only the entry but also the concentration of inorganic PO_4, SO_4, K and organic substances e.g. C14-leucine and C14-alanines in the nuclei of intact oocytes of amphibians.

Working on isolated oocyte nuclei of the amphibian, Duryee (1940, 1950), Callen (1949, 1952), Goldstein and Harding (1950), Battin (1959), Hunter and Hunter (1961), and MacGregor (1962) found water and salt solutions readily entered into the nucleus and that the swelling was

caused by osmotically active substances within the nucleus. Battin (1959) discussed various hypotheses and concluded that the colloidal osmotic pressure of the nucleoplasm was responsible for the swelling of the nucleus, while Feldherr and Harding (1964) concluded that swelling was due to the ions being absorbed within the nucleoplasm.

Although the permeability of nuclear membranes to water and salt solutions is widely accepted, workers do not agree concerning the passage of complex organic molecules. For example, the nuclear membrane is relatively impermeable to egg albumin (Goldstein and Harding 1950, Callen 1949), glycogen and acacia (Callen 1949), and bovine serum albumin (Battin 1959) and (MacGregor, 1962). On the other hand, Monne (1935) and Clark (1943) observed that dyes with varying physical properties penetrated the nucleus. Furthermore, Anderson (1953) reported that nuclease, beef serum albumin, partially hydrolysed albumin, and haemoglobin penetrate the freshly isolated liver cell nuclei. Holtfreter (1954) found frog oocyte nuclei were permeable to haemoglobin. Merriam (1959b), using an interference microscope, demonstrated that serum albumin can enter nuclei isolated from Chaetopterous eggs. Therefore, no general statement can be made concerning the permeability of larger molecules.

As no information was available on the osmotic behavior of chicken oocyte nuclei, and such information was necessary in other studies requiring isolation of the nucleus in a saline solution, a study on osmotic behaviour of the fowl oocyte nuclei was conducted.

Materials and Methods

Mature White Rock females were obtained from the University of Alberta Poultry Farm.

Ovaries containing egg follicles were aseptically dissected. Individual egg follicles were plucked and placed in a watch glass containing either .1 M (5:1) saline or a balanced experimental salt solution. Using an eyepiece micrometer fitted in a stereoscope, the follicle diameters were measured. Nuclei were isolated by opening the wall of the follicle as outlined in Isolation of Lampbrush Chromosomes (Chapter II) and the nucleus diameter immediately measured. The average diameter of the nucleus, estimated by measuring two diameters perpendicular to each other, was measured either with a magnification of 100X by an inverted microscope or a magnification of 30X under a stereoscope.

The diameter of the nucleus found in different sizes of oocytes in situ was recorded to determine the effect of different experimental salt solutions on the nuclei. Because several cellular layers must be separated from the egg follicle the isolation of the intact oocyte is rather difficult, and therefore in this section, measurements of egg follicle diameter are considered as measurements of oocyte diameters.

The experimental solutions are listed in Table 9. The isolated nuclei were placed in the solutions and the diameter measured as outlined above.

Different levels of concentration of BSA solution (Nutritional Biochemical Co., Cleveland, Ohio) were used to determine the isotonicity of the nucleoplasm.

Table 9. Solutions used in determining osmotic behavior of isolated nuclei

Experimental Solutions	Solution
Distilled water	---
KCl solution	0.05 M 0.1 M 0.5 M
Sucrose solution	0.1 M 0.5 M 0.8 M
Bovine Serum Albumin (BSA)	Solutions of 2% to 20% of Bovine Serum Albumin were prepared in 0.1 M KCl

Results

I. Relationship Between Follicle Size and Isolated Nuclear Size.

The nuclear diameters of freshly isolated nuclei are shown in Table 10 for different diameters of egg follicles. The egg follicles, ranging in size from the smallest of 0.6 mm to the largest of 9 mm, had nuclei with a minimum diameter of 195 μ and a maximum diameter of 466 μ.

The mean diameters of the nuclei isolated from the corresponding classes of egg follicles, shown in Table 10, are plotted in Fig. 174. The results indicate that as the diameter of the egg follicles increases the diameter of the nucleus increases.

The frequencies of nuclei for the nine classes of egg follicles are given in Table 11. Using these values a three dimensional model was constructed (Fig. 175). Observation of Fig. 175 shows that the frequency of the larger sized nuclei increased as growth advanced. The most predominant sized nuclei, which accounted for 80% of the isolated nuclei, were between 350 and 400 μ. Based on the results of this study, upon isolation one would expect to harvest nuclei of 250 - 300 μ diameter from egg follicles of about 1 mm diameter approximately 60% of the times, nuclei of 300 - 350 μ diameter from egg follicles of 1 - 2 mm diameter about 40% of the times, nuclei of 350 - 400 μ diameter from egg follicles of 2 - 3 mm diameter 72% of the times, and nuclei of 400 - 450 μ from isolated egg follicles of 5 - 6 mm diameter about 60% of the times.

II. Experimental Solutions.

A. Distilled Water.

The time course effects of nuclear swelling for two representative

Table 10. Minimum and maximum diameter of nuclei isolated from different sizes of egg follicles

Class	Diameter of egg follicles (mm)	No. of isolated oocytes (nuclei)	Nucleus diameter		
			Min	Max	Mean
I	0.6 - 0.9	13	195	333	279
II	1.0 - 1.9	26	195	433	296
III	2.0 - 2.9	37	200	466	340
IV	3.0 - 3.9	21	200	446	334
V	4.0 - 4.9	20	285	400	353
VI	5.0 - 5.9	20	300	420	384
VII	6.0 - 6.9	20	314	410	363
VIII	7.0 - 7.9	20	333	420	379
IX	8.0 - 8.9	25	325	466	387

Table 11. Frequency distribution of nuclei from classes of egg follicles

Size group of nuclei	Nuclei size (micron)	Egg follicle size (mm)								
		8-8.9	7-7.9	6-6.9	5-5.9	4-4.9	3-3.9	2-2.9	1-1.9	.6-.9
7	450 - 500	16.0*						2.7		
6	400 - 449	28.0	40.0	25.0	60.0	10.0	23.8	10.8		
5	350 - 399	40.0	45.0	40.0	25.0	50.0	14.3	35.1	7.7	
4	300 - 349	16.0	15.0	35.0	15.0	35.0	33.3	37.8	38.5	23.1
3	250 - 299					5.0	23.8	10.8	30.8	61.5
2	200 - 249						4.8	2.7	19.2	7.7
1	150 - 199								3.8	7.7
		IX	VIII	VII	VI	V	IV	III	II	I
		Size classes of egg follicles								

*Values are percentages of nuclei within each egg follicle size.

isolated nuclei are plotted in Fig. 176. Within 45 seconds after isolation of the nucleus in distilled water an abrupt increase in the volume of the nucleus occurred. The diameter reached a maximum in approximately 3 minutes, then regressed, and for the next 5 - 10 minutes a diffusion of nucleoplasmic elements occurred. If allowed to remain in the distilled water for longer than 10 minutes the nucleus became an empty capsule of nuclear membranes (Nuclear ghost) devoid of visible nuclear organelles and the walls become sticky.

Shortly after isolation of a nucleus a space appeared between the cloudy translucent nucleoplasmic ball, which contained chromosomes, in the center of the nucleus and the nuclear membrane. As the nucleus swelled this space became more distinct because of its transparent consistency. This sojourned two zoned condition of the nucleus became a permanent exhibit in fixed materials (Figs. 32 and 42) and was described as ecto and endonucleoplasm. After the nucleus stopped swelling the central mass moved back towards the periphery which eventually resulted in mixing of the two zones, thereby giving a homogeneous appearance.

B. KCl Solutions.

The time changes in nuclear diameter for a representative nucleus isolated in different concentrations of KCl solutions are given in Fig. 177. In very dilute salt solutions, such as 0.05 M KCl, swelling is less rapid than in distilled water. The rate of tonicity appeared to be dependent on the strength of the isolating salt solution. In solutions of very low concentrations, such as 0.01 M KCl (not shown), swelling of the nucleus was more rapid than either in a very high molar solution (0.5 - 1.0 M KCl), or in those of medium concentration (0.1 M KCl).

The nuclear tonicity in 0.05 M KCl salt solution appeared as maximum swelling within 5 minutes (Fig. 177A). Although the reaction time for the maximum swelling was similar to that observed in distilled water, the increase in the diameter of the swollen nucleus was approximately half that which occurred in distilled water.

In 0.1 M KCl the nuclear swelling peaked between 10 - 20 minutes, with a subsequent decline (Fig. 177B), while in 0.5 M KCl (Fig. 177C) the peak was reached by 15 min and then declined. The sojourned double zoned condition of the nucleus was also observed in the electrolyte solutions having different molarity. However, it was less consistently defined in KCl solutions of 0.05 - .1 M strength.

C. Sucrose Solutions.

On the basis of general observations (not shown) the swelling in a pure sucrose solution was more rapid than in KCl solution, and if the nucleus was allowed to remain in 8 - 10% pure sucrose solution for more than 1/2 hour the nuclear envelope fragmented and shreds of the nuclear wall floated to the surface. Time course of nuclear diameter isolated in different concentrations of sucrose solution prepared in 0.1 M KCl is given in Fig. 178. However, as shown in (Fig. 178A) a molar strength of 0.1 M sucrose prepared in 0.1 M KCl solution had a milder effect in that the nucleus slowly swelled and the subsequent ejection of the nuclear material was considerably delayed. The beneficial effects of an electrolyte carrier when used with sucrose may be observed by comparing it with the effect of 0.1 M KCl solution alone. Higher concentration of sucrose solution (0.8 M) in 0.1 M KCl solution showed more swelling (Fig. 178C) than lower concentration of sucrose solution

(Fig. 178A). This indicated that sucrose solution causes swelling of the nucleus, and the nuclear membrane is permeable to sucrose solution.

D. Protein.

Although Callan (1949) claimed that 15% Bovine Serum Albumin (BSA) was isotonic with amphibian oocyte nuclei, the results of Battin (1959) indicated 3 - 4% BSA to be isotonic and the results of MacGregor (1962) indicated a 6% solution of BSA was isotonic with the amphibian ooplasm. To determine the isotonic strength of BSA in fowl nuclei, crystalline BSA in 0.1 M KCl solution at pH 7 was used at different concentrations. The results are given in Table 12. Approximately 4 - 5% BSA was isotonic with chicken oocyte nucleoplasm. In concentrations less than 4 - 5% the nucleus swells after about 5 minutes and in concentrations higher than 4 - 5% the nuclei shrinks and behaves differently.

Table 12. Effect of BSA on isolated nuclei.

Experiment Number	% BSA in 0.1 M KCl Solution				
1	2% Swells*	5% No effect	8% No effect	10% Shrink	20% Shrink Burst
2	3-3.5% Swells	4-4.5% No effect	5-5.5% Flaccid	6-6.5% Shrink	7-8% Burst

*Single observation per cell.

Discussion

Isolated oocyte nuclei of amphibians and newt have been studied very extensively (Duryee 1940; Gall 1954b; Callan and Lloyd 1960; Wischnitzer 1961; Battin 1959; MacGregor 1962; and Callan 1952, 1966). The results indicate that the nucleus size increases with an increase in oocyte size. Although this is supported by the overall picture of the findings in this study, there was considerable variation in the size of the nucleus when isolated from egg follicles of the same size. This may be because in the same size egg follicles, the oocyte (cytoplasm) and the corresponding nuclei (nucleoplasm) are not in the same metabolic state. It is clear that the size of the chicken oocyte nucleus is large enough (200 - 500 μ) in the range of follicles studied, to be useful for many kinds of biological investigations.

No exact comparison with previous reports for the rate of swelling of the fowl nuclei can be made because available information on this aspect stems from other species and is subjective or qualitative (Battin 1959; MacGregor 1962; and Callan 1966), rather than quantitative as in this study. Nevertheless, the swelling of chicken oocyte nuclei in distilled water appears to be rapid. The swelling of the nucleus in distilled water always regressed after a point of maximum extension was attained. This phenomenon, called stilling, was also observed by Battin (1959) and MacGregor (1962) and it has been suggested as being due to active diffusion of the nucleoplasmic substances. Even though Battin and MacGregor suggested that this assumption could be verified by observation of nuclear membranes containing no holes, this may not be enough.

Although variation in the osmotic behaviour of nuclei was observed, particularly among nuclei of the same size egg follicles and in the same isolating medium, the observations on the tonicity of the nucleus are in close agreement with those of Duryee (1940), Callan (1949, 1952), Battin (1959) and MacGregor (1962). Battin (1959) listed several hypothesis explaining the tonicity of the nucleus: (a) expansion of the nuclear membrane, (b) hydration of the internal gel mass of the nucleoplasm, (c) permeability of the nuclear membrane to water, so that the nucleus behaves like a simple osmometer, and (d) permeability of the nuclear membrane to only small molecules and water, but not to protein and other larger molecules. Battin was convinced that swelling of the isolated nuclei was due to colloid-osmotic pressure of the nuclear sap. He assumed that differences in degree of swelling due to media of different ionic strength could be explained on the basis of Donnan's law. However, Battin's arguments against other alternatives, such as the hydration of internal gel mass of the nucleoplasm, etc., were based on inadequate experimental evidence. It is quite possible that a combination of more than one mechanism may contribute to the swelling of the nucleus.

The sojourned two zoned morphology (ectonucleoplasm and endonucleoplasm) of freshly isolated nuclei in electrolytes and distilled water was also observed by Battin (1959) and MacGregor (1962). A question is whether the rapid imbibition of water causes this sojourned clear zone in the periphery of nucleoplasm by expansion of ectonucleoplasm or extension of the prenuclar space (interlamellar space) of the nuclear wall. Evidence concerning this can be obtained by comparing the spaces in the electron micrographs of the sectioned nuclei which were isolated in the (5:1) isolating media, with the spaces in the

electron micrographs of the intact nuclei sectioned along with the fixed ovarian tissue. No difference in the prenuclear spaces of differently processed nuclei was found indicating that no expansion had taken place.

The osmotic effect of sucrose solution on nuclear swelling appears quite similar to those of Battin (1959). Tonicity of nucleus to different concentrations of experimental solution is also in agreement with Battin (1959) and MacGregor (1962).

In this experiment 4 - 5% of crystalline bovine albumin (BSA) was found to be isotonic to the isolated nucleus. This figure falls in the range of values reported by Battin (1959) and MacGregor (1962). At present, it is difficult to explain from osmotic studies on the isolated nuclei of the oocytes, the permeability of the macromolecules.

Results of osmotic behaviour on isolated nuclei reported in the literature have been quite variable. Possible reasons for such variations are: (a) the permeability characteristics of the nuclear envelope may differ due to differing sources of material, (b) during the time required for isolating the nuclei, permeability characteristics of the nuclear membrane may be altered because of irreversible chemical reactions which take place between the elements of the isolating media and the membrane, and (c) differences in the nature of the isolating media; such as pH, molarity etc.; may be responsible for determining the permeability characteristics of the nuclear membrane. However, Harding and Feldherr (1959), Feldherr and Feldherr (1960), Hunter and Hunter (1961), Feldherr (1963) and Feldherr and Harding (1964) have demonstrated that the macromolecules of 40,000 M.W. or larger do not freely penetrate the nuclear envelope of amphibian oocyte. Using interferometry,

MacGregor (1962), working on the nuclear mass, also concluded that the amphibian oocyte nuclear membrane was freely permeable to water and salts but not protein.

The present findings, although based on limited data, are in agreement with those of the above workers and suggests that for osmotic properties the chicken oocyte nucleus behaves in a manner identical with the amphibian oocyte nucleus. The similarity between the fowl oocyte and amphibian oocyte nuclei is not only in their osmotic behaviour but also in the ultrastructural morphology of the nuclear envelope. However, a few characteristic differences between the amphibian and avian oocyte nuclei which were observed in the isolation experiments should be re-emphasized: (a) Unlike amphibians, avian oocytes are encapsulated with a number of thick follicular layers so that the isolation of the nucleus is relatively difficult, (b) In fowl, the oocyte nuclei has a liquid gel nucleoplasm and has never been observed in a stiff gel condition except when kept in refrigerator for several hours or days, (c) Nuclei of the fowl are less transparent than amphibian oocyte nuclei, and (d) Although the size of oocyte nuclei may be as large as 600 μ in egg follicles above 1 cm., isolation of nuclei from larger than 6 mm dia egg follicles is difficult because the nuclear wall becomes less manageable due to the stickiness and rough folded surface.

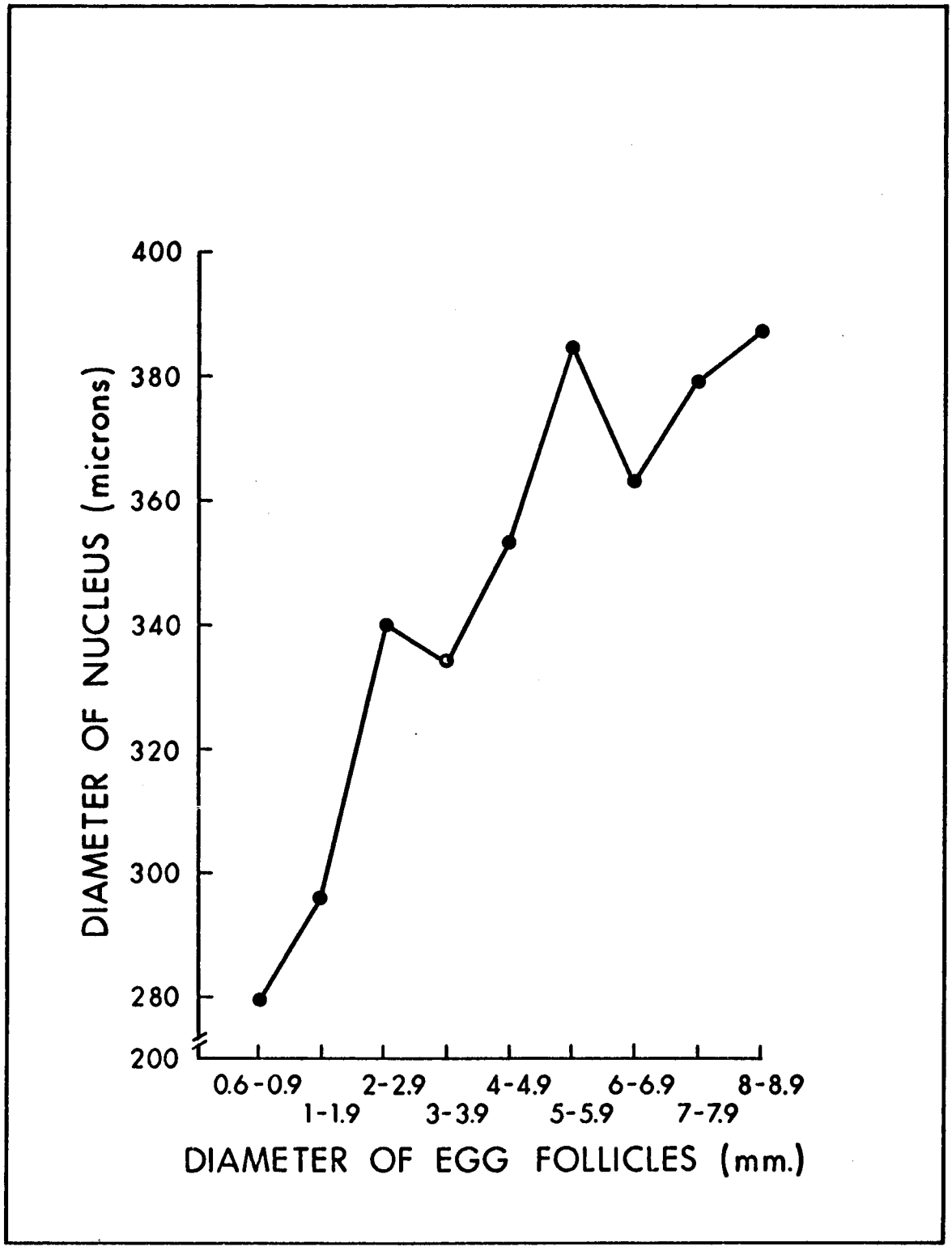


Fig. 174. Size of nuclei isolated from different size egg follicles.

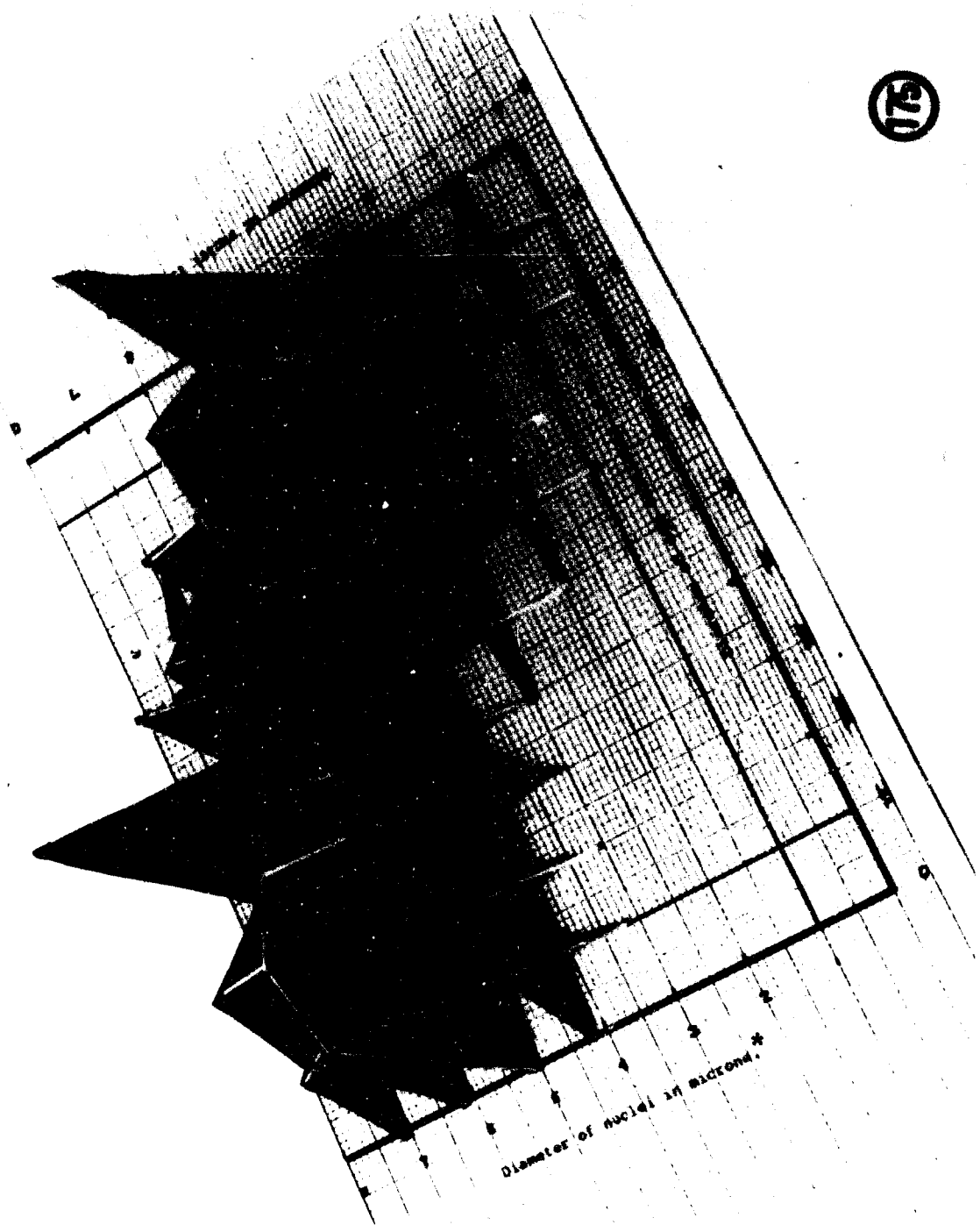
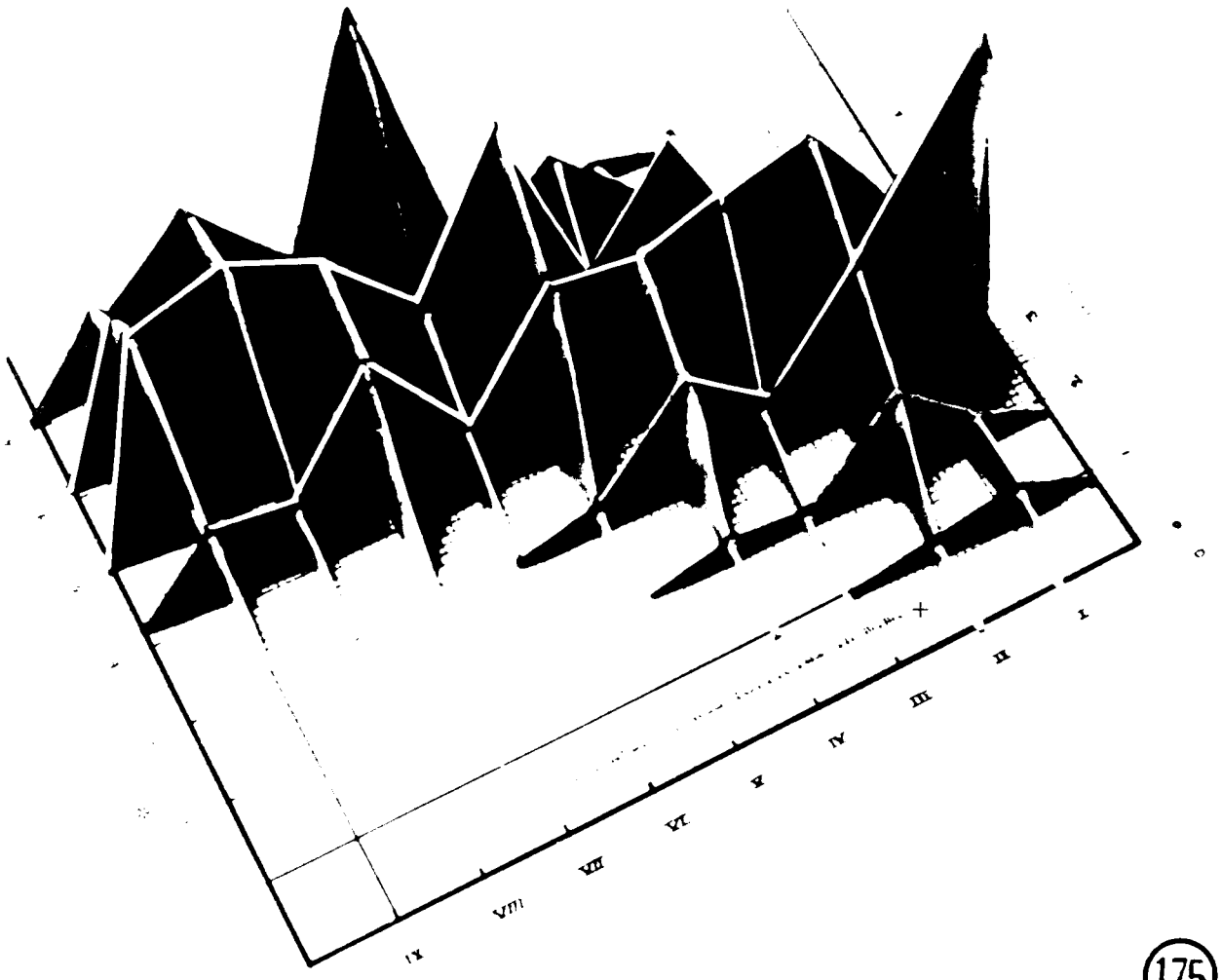


Fig. 175. Three dimensional model showing percentage of each size group of nuclei occurring within each size class of egg follicles (* Groups of different size intervals; + Classes of different size intervals see Table 11).


IX
VIII
VII
VI
V
IV
III
II
I

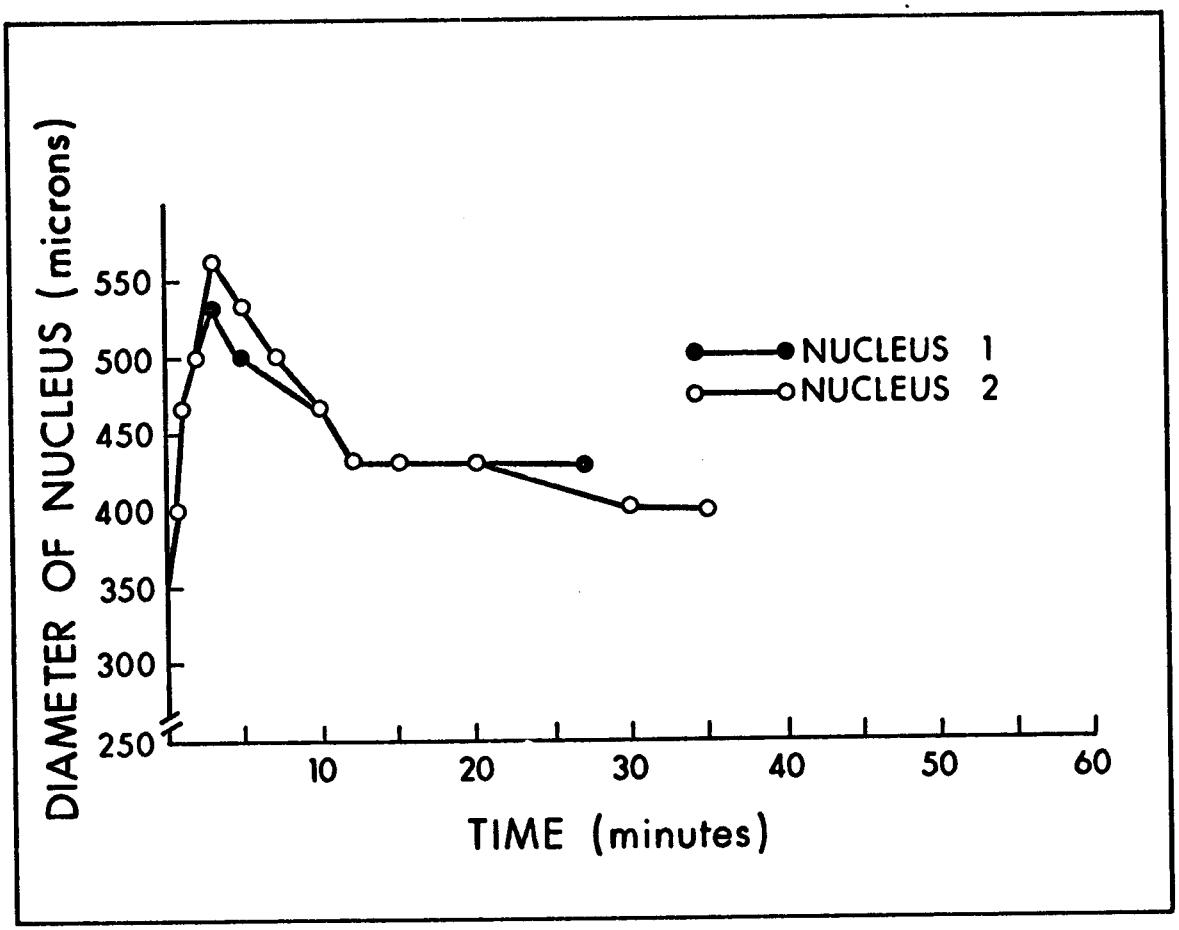


Fig. 176. Time course of nuclear diameter for two representative nuclei isolated in distilled water.

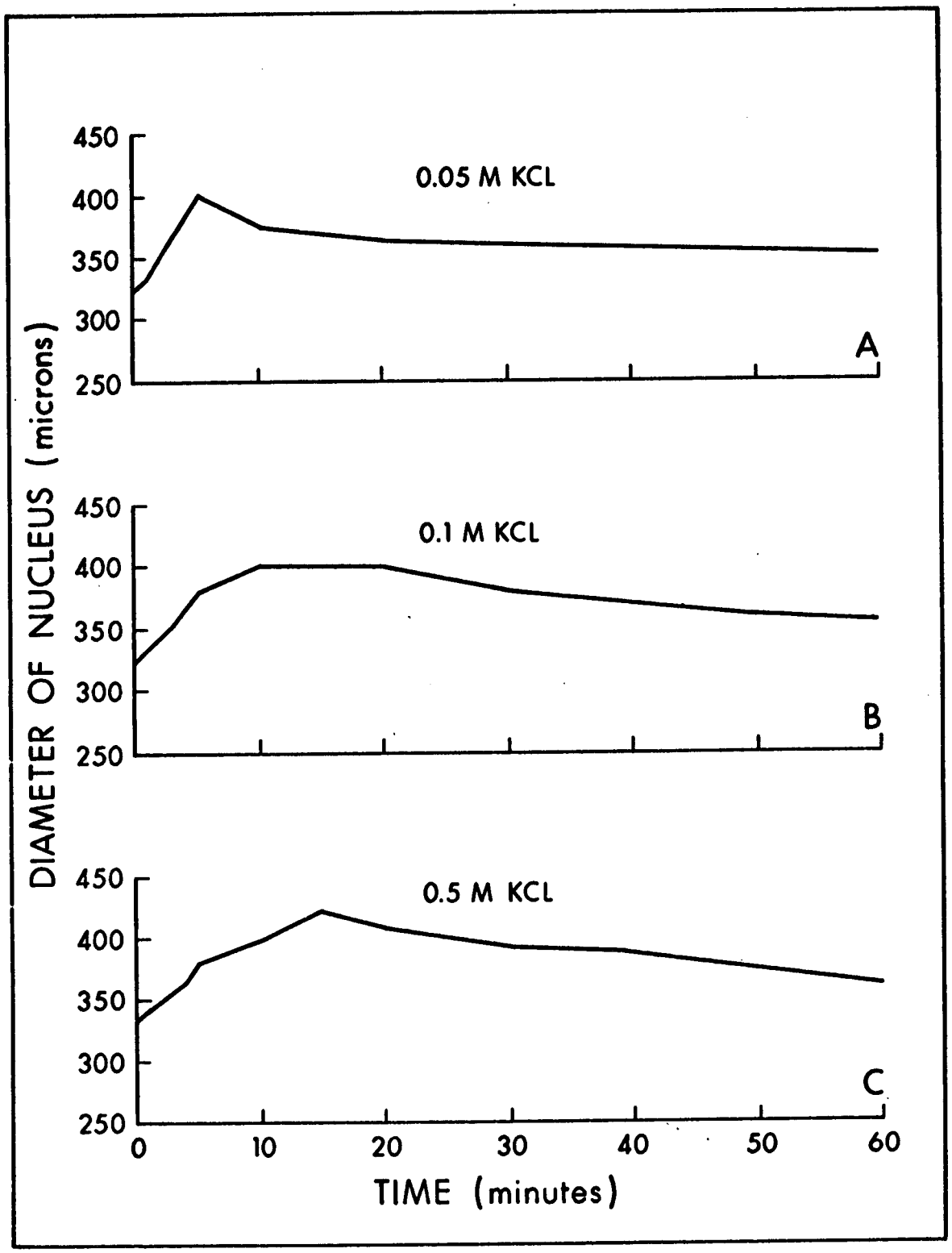


Fig. 177. Time course of nuclear diameter for a representative nucleus isolated in different concentration of KCl solutions.

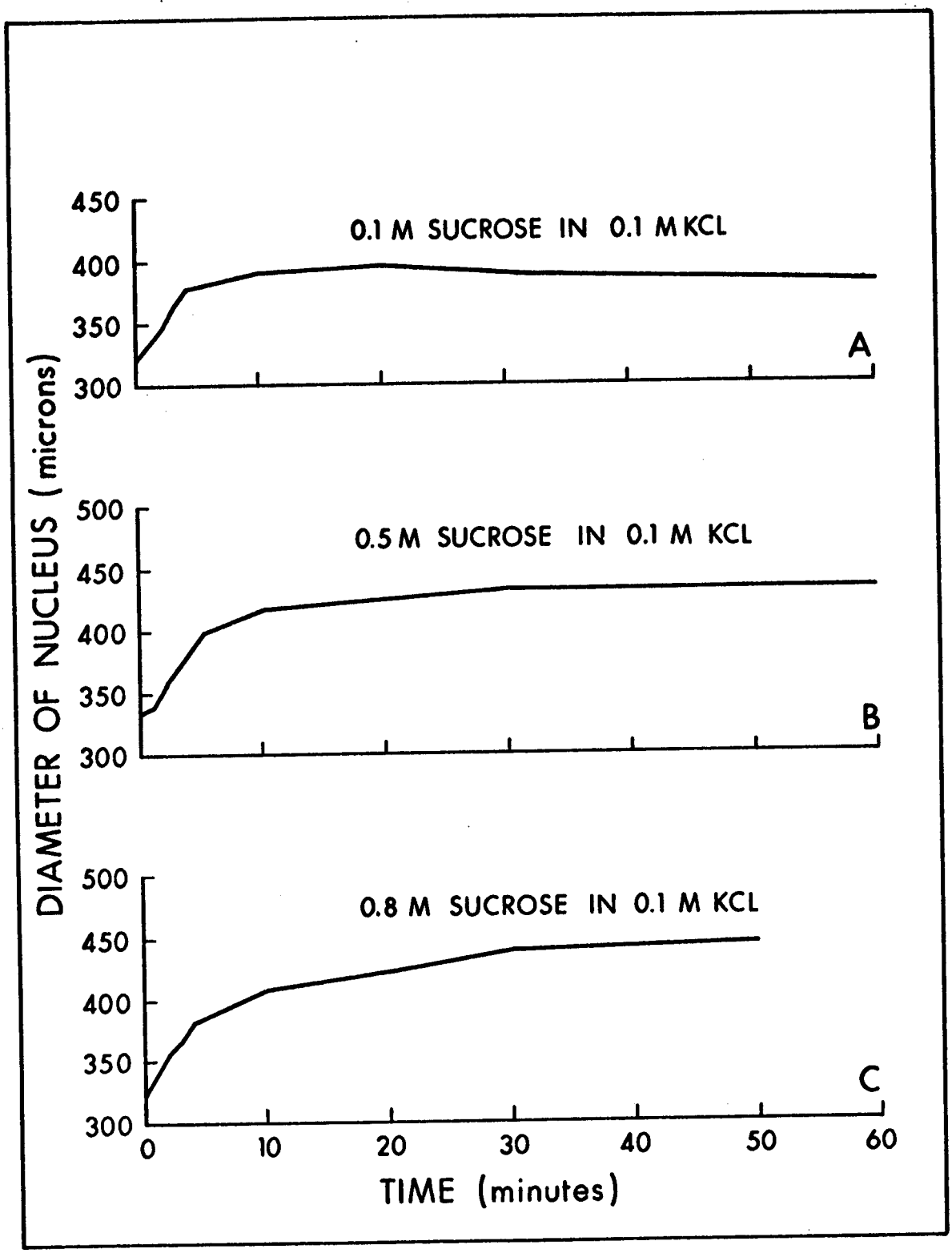


Fig. 178. Time course of nuclear diameter for representative nuclei isolated in 0.1 KCl-Sucrose solutions.

SUMMARY AND CONCLUSIONS

The diploid number of Gallus domesticus metaphase chromosomes obtained from dividing feather follicle cells is in the upper seventies. Although a new mitotic agent (podophyllin) was also used, no exact count could be obtained because the smallest of the micro-chromosomes are beyond the limits of the light microscope. Contrary to Chromosomoid hypothesis of Newcomer, the evidence indicated that the microchromosomes behave normally and are similar to macrochromosomes in all respects during mitosis and enzyme action. In future studies, additional information concerning the metaphase chromosomes of domestic fowl may be obtained by use of scanning electron microscope of a mono-layer spread of mitotic plates.

A technique for the manual isolation of oocyte nuclei and its contents (lampbrush chromosomes and nucleoli) was developed. The nuclei isolated from egg follicles (0.6 mm - 1 cm) varied from 200 to 500 U in diameter. The nucleus of fowl oocyte is easy to isolate and is a source for further cytogenetic investigations.

Osmotic response of isolated nuclei to KCl and sucrose solutions was identical to those described for amphibians. In distilled water the nuclei swelled to a maximum diameter within three minutes. The nucleoplasm of the isolated nuclei appears to be isotonic to 4 - 5% Bovine Serum Albumin solution.

Not only does the fowl oocyte resembles amphibian oocyte nuclei in osmotic behaviour, but also in the ultrastructural morphology of the nuclear envelope. As in newt, frog, and starfish the nuclear envelope of the fowl oocyte is bilamellar and contains nucleopores supported by

annuli which contain, inconsistently, central granules. Each lamella of the envelope, 100 - 125 Å thick, is separated by a 175 - 200 Å interlamellar space. Approximately 15% of the nuclear envelope is covered by electron dense rings annuli 1000 Å, which supports the nucleopores (600 - 800 Å) on either side of the envelope. The annuli are highly complex structures whose fine organization and nature is neither well defined or known. The nuclear envelope of oocytes which contains nuclei larger than 50 μ becomes folded and/or corrugated and the nucleoplasmic gel becomes bipartite consisting of ecto- and endonucleoplasm. In still larger oocytes the outer nuclear membrane forms blebbs and cytoplasmic annulate lamellae. Observation of electron micrographs of the nucleocytoplasmic regions suggest the passage of nuclear microvesicles across the nuclear wall.

Light microscopy revealed that irrespective of age after 18 days and above 150 μ dia the active oocytes contained lampbrush chromosomes. In the remainder of the oocytes, both in growing and laying fowls, the resting oocytes contained chromatin clumps or reticulum while the less active ones contained chromosomes in diplotene, with or without a large nucleolus. In advanced oocytes, larger than 500 μ dia, the nucleoplasm becomes granular and later contains hundreds of chromatic and peripheral nucleoli. Before transforming to lampbrush stage, diplotene chromosomes become polar or parallel to periphery of the nucleus and their axes appear segmented due to excessive enlargement of the chromomeres. The chromomeres spin out the lateral loops on either side of the chromosomes.

The process of transformation of the chromosomes from diplotene

to lampbrush configuration appears to be associated with the activity of the Balbiani body and centriole in the cytoplasm. Furthermore, the transformation of lampbrush chromosomes seems synchronous, at least in the macrochromosomes, as in a developed oocyte (700 μ) at least 12 lampbrush chromosomes could be observed in one nucleus. The lateral loops of the lampbrush chromosomes, both in the stained section preparations as well as in isolated nuclei were studded with chromatic nucleoli (granules) in a single linear array. Egg folicles from 0.6 mm to 3 mm dia usually contained up to 3 or 4 typical isolatable lampbrush bivalents per nucleus while in egg follicles above 3 mm dia the nuclei yielded only nucleolar lampbrush chromosomes or a reticular network of heteromorphic fibers (lampbrush chromosome elements). At a later stage the intact isolated nuclei were filled with ribbon-like fine strands of chromosomes attached with chromatic nucleoli and/or contained chromatic reticulum. This stage stays until the formation of clumped masses before the reduction division.

The information obtained in this study suggests that the breakdown of lampbrush chromosomes is asynchronous and also that the lampbrush chromosome is further modified by overextending its lateral loops and unfolding its axes into finer basic strands of 250 Å thick so that a diplotene or diakenetic configuration of chromosomes is no longer identifiable. These unfolded chromosomal strands become involved in extensive synthesis of nucleoli, i.e. the nucleolar RNA. Thus this stage of chromosomes is analogous to that of the somatic interphase chromosome which unfolds to finer nucleoprotein fibers and is involved in replication of chromosomal DNA. Because the fowl oocyte contains a large amount of egg protein and other cytoplasm inclusions it is possible

that the pattern of lampbrush chromosome organization in the domestic fowl may be slightly different from amphibian and fishes.

Isolated nucleoli (solid spheroid, beaded ring, and pearl string) appear similar to those described for other animal oocytes. Giant nucleoli, 50 to 150 μ dia, are present, inconsistently, in laying hen egg follicles of 0.6 to 2.5 mm dia. Occasionally attached to the giant nucleoli are a cluster of fibers which may be the lampbrush stage of microchromosomes. Incorporation of uridine H^3 indicated that RNA was concentrated in the core of the giant nucleoli with a gradual centrifugal decrease in the attached fibers.

The unfolding of the chromosome strands (post-lampbrush stage) with a simultaneous excessive production of oocyte nucleoli appears to be associated with the disappearance of the giant nucleolus. The results of this project suggests several areas of investigation, for example:

1) study of nucleocytoplasmic exchanges.
2) study of nucleolar gene by autoradiography of lampbrush chromosome using both light and electron microscopy.
3) isolation of oocytes less than 1 mm dia which may yield the lampbrush bivalent stage of all chromosomes in haploid number.
4) nature of giant nucleolus.

BIBLIOGRAPHY

Abelson, P.H. and W.R. Duryee. 1949. Radioactive sodium permeability and exchange in frog eggs. Biol. Bull. 96: 205-217.

Afzelius, B.A. 1955. The ultrastructure of the nuclear membrane of the sea urchin oocyte as studied with the electron microscope. Exptl. Cell Res. 8: 147-158.

Akkeringa, L.T. 1927. Die Chromosomen bei einigen Huhnerrassen. Z. Mickr. Anat. Forsch. 8: 325-342.

Anderson, E. 1968. Cortical alveoli formation and vitellogenesis during oocyte differentiation in the Pipefish, Syngnothus fuscusi and the Killifish, Fundulus hete joclitus. J. Morph. 125: 23-60.

Anderson, N.G. 1953. On the nuclear envelope. Science. 117: 517.

Bajer, A. 1965. Subchromatid structure of chromosomes in the living state. Time lapse photography. Chromosoma. 17: 291-302.

Bajer, A. and R.D. Allan. 1966. Structure and organization of the living mitotic spindle of Haemanthus endosperm. Science 151: 572-574.

Baker, T.G. and L.L. Franchi. 1967. The structure of chromosomes in human primordial oocytes. Chromosoma 22: 358-377.

Bal, A.K., F. Jubinville and G.H. Cousineau. 1969. Nuclear changes during meiotic prophases of Arbacia punctulata primary oocytes as observed by electron microscopy and autoradiography. Paper read at the 12th Annual Canadian Federation of Biological Societies, Edmonton, Alberta, June 13, 1969.

Bammi, R.K., R.N. Shoffner and G.J. Haiden. 1966. Sex chromosomes in germ cells of the chicken, turkey, Japanese quail. Poult. Sci. 45: 424-426.

Barnes, B.G. and J.M. Davis. 1959. The structure of nuclear pores in mammalian tissue. J. Ultrastruct. Res. 3: 131-146.

Battin, W.T. 1959. The osmotic properties of nuclei isolated from amphibian oocytes. Exp. Cell Res. 17: 59-75.

Beams, H.W. 1964. Cellular membrane in oogenesis. Symp. Devel. Growth 22: 175-219.

Beams, H.W. and R.G. Kessel. 1963. Electron microscope studies on developing crayfish oocyte with special reference to the origin of yolk. J. Cell Biol. 18: 621-649.

Beck, L.V. and H. Shapiro. 1936. Permeability of germinal vesicle of Starfish eggs to water. Proc. Soc. Exp. Biol. 34: 170-172.

Belkin, M. 1947. Effect of podophyllin on transplanted mouse tumors. Fed. Proc. 6: 308.

Bellair, R. 1965. The relationship between oocyte and follicle in the hen's ovary as shown by electron microscopy. J. Embryol. Exp. Morph. 13: 215-233.

Bernhard, W. 1966. Ultrastructural aspect of normal and pathological nucleolus in mammalian cells. Nat. Cancer Inst. Monogr. 23: 13-78.

Bier, K., K. Kunz and D. Ribbert. 1967. Struktur und Funktion der Oocytenchromosoma und Nukleolen sowie der Extor-DNS. waehrend der Oogenese Panoistischer ameroistischer Insekten. Chromosoma 23: 214-254.

Bloom, S.E. and E.G. Buss. 1967. A cytological study of mitotic chromosomes in chicken embryos. Poult. Sci. 46: 518-527.

Bloom, S.E. and E.G. Buss. 1968. The effect of age and kind of tissue on mitotic activity in chicken embryos. Poult. Sci. 47: 837-839.

Boring, A.M. 1923. Notes on chromosomes of the domestic chicken. Science 58: 73-74.

Boring, A.M. and R. Pearl. 1914. The odd chromosome in the spermatogenesis of the domestic chicken. J. Exp. Zool. 16: 53-84.

Borisy, C.C. and E.W. Taylor. 1967a. The mechanism of action of colchicine. J. Cell Biol. 34: 535-548.

Borisy, C.C. and E.W. Taylor. 1967b. The mechanism of action of colchicine. Binding of colchicine H^3 to cellular protein. J. Cell Biol. 34: 525-533.

Bovey, R. 1952. Electron microscopy and micro dissection. J. R. Micr. Soc. 72: 56-63.

Brachet, J. 1929. Recherches le comportément de l'acide thymonucléinique au cours de l'oogénèse chez diverses éspèces animales. Arch. Biol. (Paris). 39: 678-697.

Brachet, J. 1940. La localisation de l'acide thymonucléique pendant l'oogénèse et la maturation chez less Amphibiens. Arch. Biol. (Paris). 51: 151-165.

Brachet, J. 1942. La localisation des acides pentosenucléiques dans les tissus animaux et less oeufs d'amphibiens en voie de développement. Arch. Biol. (Liège). 53: 207-257.

Brambell, F.W.R. 1925. Oogenesis of the fowl Gallus bankiva. Phil. Trans. R. Soc., B. 214: 113-151.

Brant, J.W.A. 1952. A review of the literature on chromosome studies in the fowl. Poult. Sci. 31:409-417.

Brink, J.M. van. 1959. L'expression morphologique de la digametie chez les Sauropsides et les Monotremes. Chromosoma 10: 1-72.

Brink, J.M. van. and G.A. Ubbels. 1956. La question des hétéro-chromosomes chez les Sauropsides. Oiseaux. Experientia. 12: 162-164.

Brown, C.A. and H. Ris. 1959. Amphibian oocyte nucleoli. J. Morph. 104: 377-402.

Brown, D.D. 1966. The nucleolus and synthesis of r RNA during oogenesis and embryogenesis of Xenopus levis. Nat. Cancer Inst. Monogr. 23: 297-309.

Brown, D.D. and J. Gordon. 1964. Absence of r RNA synthesis in anucleolate mutant of Xenopus lavis. Proc. Nat. Acad. Sci. U.S.A. 51: 139-146.

Brown, R. 1883. Observations on the organs and mode of feconvation in Archideae. Trans. Linn. Soc., 16:685. Cited by Stevens, A.R. 1966.

Buck, J.B. and R.D. Boche. 1938. Some properties of living chromosomes. Biol. Bull. 75: 344. (Abstract).

Callan, H.G. 1949. A physiological study of isolated nuclei and its implication regarding gene action. Heriditas (Suppl). 35:547-548.

Callan, H.G. 1952. A general account of experimental work on amphibian oocyte nuclei. Proc. Soc. Exptl. Biol. 6: 243-255.

Callan, H.G. 1955. Recent work on the structure of cell nuclei, pp. 89-109. Symposium on the Fine Struct. of Cells (Leiden). Interscience Publishers, New York.

Callan, H.G. 1957. The lampbrush chromosomes of Sepia officianalis L., Anilocra physodes L. and Scyllium catulus cuv; and their structural relationship to the lampbrush chromosomes of amphibian. Publ. Staz. Zool. Napoli. 29: 329-346.

Callan, H.G. 1959. Organization of the chromosome, pp. 42-87. Biological Organization. Edited by C.H. Waddington. Pergamon Press, Edinburgh.

Callan, H.G. 1963. The nature of lampbrush chromosomes. Int. Rev. Cytol. 15: 1-34.

Callan, H.G. 1966. Chromosome and nucleoli of axolotl, Ambystoma mexicanum. J. Cell Sci. 1: 85-107.

Callan, H.G. and L. Lloyd. 1960. Lampbrush chromosomes of crested newts, Triturus cristatus (laurenti). Phil. Trans. R. Soc., B. 243: 135-219.

Callan, H.G. and H.C. MacGregor. 1958. Action of DNase on lampbrush chromosomes. Nature 181: 1479-1480.

Callan, H.G. and S.G. Tomlin. 1950. Experimental studies on amphibian oocyte nuclei. I. Investigation of the structure of the nuclear membrane by means of the electron microscope. Proc. Roy. Soc., B. 137: 367-378.

Carnoy, J.B. and H. Lebrun. 1897. La vesicule germinative et les globules polaires chez les Batraciens. Cellule 12: 191-295.

Carr, D.H. and J.E. Walker. 1961. Carbol fuchsin as a stain for human chromosomes. Stain Technol. 36: 233-236.

Chamber, R. and H.B. Fell. 1931. Micro-operations on cells in tissue cultures. Proc. Roy. Soc., B. 109:380-403.

Choudhary, H.S. 1951. Nucleolar activity in the oocytes of some marine Teleostean fishes. J.R. Micr. Soc. 71: 87-93.

Chouinard, L.A. 1963. Sites of formation of the extra nucleoli during early oocyte growth in the freshwater Teleost salvelinus fontinalis mitchill. Canad. J. Zool. 41: 997-1010.

Churney, L. 1942. The osmotic properties of the nucleus. Biol. Bull. 82: 52-67.

Cole, A. 1962. A molecular model for biological contractility: Implications in chromosome structure and function. Nature 196: 211-214.

Cole, A. 1967. Chromosome structure, pp. 305-375. Theoretical and Experimental Biophysics. Edited by A. Cole. Marcel Dekker, New York.

Coleman, J.R. 1964. Fine structure and cytochemistry of the meiotic chromosome of domestic rooster. Diss. Abstr. 25: 13184.

Coleman, J.R. and M.J. Moses. 1964. DNA and fine structure of synaptic chromosomes in domestic roosters, Gallus domesticus. J. Cell Biol. 23: 63-78.

Colombo, G.P. 1955. Sulla structura microscopica dei cromosomi. Experientia. 11: 333-339.

Cornman, I. and M.E. Cornman. 1951. The action of podophyllin and its fraction on marine eggs. Ann. N.Y. Acad. Sci. 51: 1443-1481.

Darlington, C.D. 1955. The chromosome as a physico-chemical entity. Nature 176: 1139-1144.

Dodson, E.O. 1948. A morphological and biochemical study of lampbrush chromosomes of vertebrates. Univ. Calif. Publs. Zool. 53: 281-314.

Dodson, E.O. 1953. Nucleoli and the formation of yolk in the eggs of vertebrates. J.R. Micr. Soc. 72: 177-178.

Donnelly, G.M. 1962. Cytological studies in domestic fowl. Ph.D. Thesis. University of Connecticut, Storrs.

Donnelly, G.M. and E.H. Newcomer. 1963. Autoradiographic patterns in cultured leucocytes of the domestic fowl. Exp. Cell Res. 30: 363-368.

Du Praw, E.J. 1965. Macromolecular organization of nuclei and chromosomes: A folded fiber model based on whole mount electron microscopy. Nature 206: 338-343.

Du Praw, E.J. 1966. Evidence for a folded fiber organization on human chromosomes. Nature 209: 577-581.

Durme, M. van. 1914. Nouvelles recherches sur le vitellogenese des oeufs d'oiseaux aux stades d'accroisserment de maturation de fecondation et du debut de la segmentation. Archs. Biol. (Paris). 29: 71-198.

Duryee, W.R. 1937. Isolation of nuclei and nonmitotic chromosome pairs from frog eggs. Arch. Exp. Zellforsch. 18: 171-176.

Duryee, W.R. 1940. Permeability of the nuclear membrane evidenced by intra nuclear colloidal changes. Proc. Eighth. Amer. Scientific Congr. 3: 45.

Duryee, W.R. 1941. The chromosomes of amphibian nucleus, pp. 129-141. Cytology, Genetics and Evolution. University of Pennsylvania Press, Philadelphia.

Duryee, W.R. 1950. Chromosome physiology in relation to nuclear structure. Ann N.Y. Acad. Sci. 50: 920-953.

Ebstein, B.S. 1967. Tritiated Actinomycin D. as a cytochemical label for small amount of DNA. J. Cell Biol. 35: 709-712.

Elizabeth, R.M. 1968. Ribonucleoprotein particles in amphibian oocyte nucleus: possible intermediate in ribosome synthesis. J. Cell Bio. 36: 421-432.

Estable, C. 1966. Preface. Nat. Cancer Inst. Monogr. 23: VX-XVI.

Evans, H.J. 1967. The nucleolus, virus infection and trisomy in man. Nature 214: 361-363.

Fankhauser, G. and R.R. Humphrey. 1963. The relation between number of nucleoli and number of chromosome sets in animal cells. Proc. Nat. Acad. Sci. U.S.A. 29: 366-380.

Feldherr, C.M. 1963. A possible regulatory mechanism for nucleo-cytoplasmic exchanges. Fed. Proc. 22: 179.

Feldherr, C.M. and A.B. Feldherr. 1960. The nuclear membrane as a barrier to the free diffusion of proteins. Nature 185: 250-251.

Feldherr, C.M. and C.V. Harding. 1964. The permeability characteristics of nuclear envelope at interphase. Protoplasmatologia 5: 35-49.

Fieq, A. 1955. Élude autoradiographique du métabolism de l'oocyte d'Asterias rubiens au cours de la croissance. Arch. Biol. (Paris). 66: 509-524.

Flemming, W. 1882. Zellsubstanz, Kern and Zellteilung. Leipzig. Cited by E.O. Dodson 1948.

Folliot, R. 1968. Intra nuclear annulate lamellae in the male germinal tissue of the insect, Philaenus spumarius (Homoptera). Z. Zellforsch 92: 115.

Fontana, F. 1781. Cited by C. Estable, 1966.

Ford, E.H.R. and D.H.M. Woollam. 1964. Testicular chromosomes of Gallus domesticus. Chromosoma 15: 568-578.

Franceschini, M.P. and A. Santoro. 1965. L'ultrastructura dell 'epitelio amniotico di embrione di pollo, migrante in coltura. Bull. Soc. Ital. Biol. Sper. 41: 619-622.

Franchi, L.L. and A.M. Mandl. 1962. The ultrastructure of oogonia and oocytes in the foetal and neonatal rats. Proc. R. Soc., B. 157: 99-114.

Franke, W.W. 1966. Isolated nuclear membrane. J. Cell Biol. 31: 619-623.

Frederic, J. 1961. Contribution à l'étude du caryotype chez le poulet. Arch. Biol. (Paris). 72: 185-209.

Gall, J.G. 1954a. Lampbrush chromosomes from oocyte nuclei of the newt. J. Morp. 94: 283-351.

Gall, J.G. 1954b. Observation on the nuclear membrane with electron microscope. Exp. Cell Res. 7: 197-200.

Gall, J.G. 1956. On the submicroscopic structure of chromosomes. Brookhaven Symp. Biol. 8: 17-32.

Gall, J.G. 1958. Chromosomal differenciation, pp. 103-135. Chemical Basis of Development. Edited by W. McElroy and B. Glass. The Johns Hopkins Press, Baltimore.

Gall, J.G. 1959. The nuclear envelope after $KMnO_4$ fixation. J. Biophys. Biochem. Cytol. 6: 115-117.

Gall, J.G. 1962. Personal Communication. Cited by J.H. Taylor, 1963.

Gall, J.G. 1963. Kinetics of DNase action on chromosomes. Nature 198: 36-38.

Gall, J.G. 1964. Electron microscopy of the nuclear envelope. Protoplasmatologia 5: 4-25.

Gall, J.G. 1966a. Techniques in the study of lampbrush chromosomes, pp. 37-60. Methods in Cell Physiology, Vol. II., edited by D.M. Prescott. Academic Press, New York.

Gall, J.G. 1966b. Chromosome fibers studies by a spreading technique. Chromosoma 20: 221-233.

Gall, J.G. 1966c. Nuclear RNA of the salamander oocyte. Synthesis of RNA in nucleolus and its transfer to cytoplasm. Nat. Cancer Inst. Monogr. 23: 475-488.

Gall, J.G. 1967. Octagonal nuclear pores. J. Cell Biol. 32: 391-399.

Gall, J.G. and H.G. Callan. 1962. H^3-Uridine incorporation in lampbrush chromosomes. Proc. Nat. Acad. Sci. U.S.A. 48: 562-570.

Gay, H. 1956. Chromsome-nuclear membrane - cytoplasmic interrelation in Drosophila. J. Biophys. Biochem. Cytol. (Suppl). 2: 407.

Gelfant, S. 1963. Inhibitors of cell division: A critical and experimental analysis. Int. Rev. Cytol. 14: 1-34.

Gersch, M. 1940. Untersuchungen uber die Bedeutung der Nucleolen im Zellkern. Z. Zellforsch. 30: 483.

Gimenez-Martin, G. and J.F. Lopez-Sacz. 1965. Chromosome structure in the course of mitosis. Cytologia (Tokyo). 30: 14-22.

Goldsmith, J.B. 1928. The history of germ cells in the domestic fowl. J. Morph. 46: 275-300.

Goldstein, L. and C.V. Harding. 1950. Osmotic behaviour of isolated nuclei. Fed. Proc. 9: 48-49.

Greenfield, M.L. 1966. The oocyte of the domestic chicken shortly after hatching, studied by electron microscopy. J. Embryol. Exp. Morph. 15: 279-316.

Guyenot, E. and M. Danon. 1953. Chromosomes et ovocytes des Batriciens. Rev. Suisse Zool. 60: 1-129.

Guyer, M.F. 1909. The spermatogenesis of domestic chicken Gallus gallus domesticus. Anat. Anz. 34: 573-580.

Guyer, M.F. 1916. Studies on the chromosomes of the common fowl as seen in testes and embryos. Biol. Bull. 31: 221-268.

Hamburger, J. 1902. Osmotischer Druck and Ionenlehre. 3 Vols. Wiesbaden. Cited by H.C. MacGregor, 1962.

Hamburger, V. 1960. A Manual of Experimental Embryology. University of Chicago Press, Chicago.

Hance, R.T. 1924. Somatic chromosomes of the chick and their possible sex relations. Science 59: 424-425.

Hance, R.T. 1926. Sex and the chromosomes of the domestic fowl. J. Morph. 43: 119-136.

Harding, C.V. and C. Feldherr. 1959. Semipermeability of the nuclear membrane in the intact cells. J. Gen. Physiol. 42: 1155-1165.

Hartmann, J.F. 1953. An electron optical study of sections of central nervous system. J. Comp. Neurol. 99: 201-227.

Holl, M. 1890. Ueber die Reifung der Eizelle des Huhn's. Sitzungsber. Akad. Wiss. Wien, Math. -Nat. Kl. 99:311-370. Cited by G.M. Donnelly, 1962.

Hollander, d.F. 1904. Recherches sur l'oogénèses et sur la structure et al signification du noyau vitellin de Balbiani chez les oiseaux. Arch. Anat. Micr. 7: 117-176.

Holtfreter, V. 1954. Observation on the physico-chemical properties of isolated nuclei. Exp. Cell Res. 7: 95-102.

Hsu, W.S. 1963. The nuclear envelope in the developing oocytes of tanicate, Baltinia villosa. Z. Zellfrosch. 58: 660-678.

Hughes, G.C. 1963. The population of germ cells in the developing female chick. J. Embryol. Exp. Morph. 11: 513-536.

Hunter, A.S. and F.R. Hunter. 1961. Studies of volume changes in the isolated amphibian germinal vesicle. Exp. Cell Res. 22: 609-618.

Inoue, S. and H. Sato. 1963. Arrangement of DNA in living sperm: A biophysical analysis. Science 136: 1122-1124.

Jorgensen, H. 1913. Morphologische Beitraege zum Problem des Eiwachstums. Arch. Zellfrosch. 10: 1-126.

Kaplan, I.W. 1942. Condylomata acuminata. New Orleans Med. Surg. J. 94: 388-390. Cited by S. Makino and T. Tanaka, 1953.

Kauffmann, B.P. 1948. Chromosome structure in relation to the chromosome cycle. Bot. Rev. 14: 57-126.

Kessel, R.G. 1964. Intra nuclear annulate lamellae in oocytes of tunicate Steyela partita. Z. Zellforsch. 63: 37.

Kessel, R.G. 1965. The intra nuclear and cytoplasmic annulate lamellae in oocytes. J. Cell Biol. 24: 471-427.

Kezer, J. 1965. Cited by W.J. Peacock, 1965.

King, L.S. and M. Sullivan. 1946. The similarity of the effect of podophyllin and colchicine and their use in the treatment of Condylomata acuminata. Science 104: 244-245.

King, L.S. and M. Sullivan. 1947. Effect of podophyllin and of colchicine on normal skin, on Condyloma acuminatum. Arch. Path. 43: 374-386.

Koecke, H.U. and K.M. Muller. 1965. Formwechsel and Anzahl der Chromosomen bei Huhn and Ente. Naturwissenschaften 52: 483.

Koltzoff, N.K. 1938. The structure of chromosomes and their participation in cell metabolism. Biol. Zh. Mosk. 7: 3-46.

Krishan, A. 1962a. Microchromosomes in embryonic mitosis of domestic fowl. Experientia 18: 365-367.

Krishan, A. 1962b. Avian microchromosomes; as shown by prefixation treatment in colchicine, squashing and hematoxylin staining. Stain Technol. 37: 335.

Krishan, A. and R.N. Shoffner. 1966. Sex chromosome in domestic fowl, (Gallus domesticus), turkey (Meleagris gallopavo), and the chinese pheasant (Phasianus colchicus). Cytogenetics 5: 53-63.

Kunz, W. 1967. Lampbrush chromosomes and multiple nucleoli in orthopteren. Chromosoma 21: 446-462.

Lafontaine, J.G. and H. Ris. 1958. An electron microscope study of lampbrush chromosomes. J. Biophys. Biochem. Cytol. 4: 99-106.

Lane, N.J. 1967. Spheroidal and ring nucleoli in amphibian oocytes: Pattern of Uridine incorporation and fine structural features. J. Cell Biol. 35: 421-434.

Leiter, J., V. Downing, J.L. Hartwell and M.J. Shear. 1950. Damage induced in sarcoma 37, with podophyllin, podophyllotoxin, and alpha-pelatin, beta-peltatin and quercetin. J. Nat. Cancer Inst. 10: 1273-1293.

Levine, M. 1951. The action of colchicine on cell division in human cancer, animal and plant tissue. Ann. N.Y. Acad. Sci. 51: 1365-1408.

Loyez, M. 1906. Recherches sur le dèveloppement ovarien des oeufs méroblastiques à vitellus nutritif abondant. Arch. Anat. Micr. 8: 69-397.

Luft, J.H. 1956. Permanganate -- a new fixative for electron microscopy. J. Biophys. Biochem. Cytol. 2: 799-801.

MacGregor, H.C. 1962. The behaviour of isolated nuclei. Exp. Cell Res. 26: 520-528.

MacGregor, H.C. 1965. The role of lampbrush chromosome in the formation of nucleoli in amphibian oocytes. Quart. J. Micr. Sci. 106: 215-228.

MacGregor, H.C. and H.G. Callan. 1962. The action of enzyme on lampbrush chromosomes. Quart. J. Micr. Sci. 103: 173-203.

Makino, S. and T. Tanaka. 1953. The cytological effect of chemicals on ascites sarcomas. 1. Partial damage in tumor cells by temporary regression and prolongation of life of tumor-bearing rats. J. Nat. Cancer Inst. 13:1185-1191.

Marechal, J. 1907. Sur l'ovogénèse des Selaciens et de quelques autres Chordates. Cellule 24: 7-239.

Marza, V.D. and R.V. Marza. 1935. The formation of the hen's egg. Quart. J. Micr. Sci. 78: 134-189.

Merriam, R.W. 1959a. The origin and fate of annulate lamellar in maturing sand dollar eggs. J. Biophys. Biochem. Cytol. 5: 117-121.

Merriam, R.W. 1959b. Permeability and structural characteristics of isolated nuclei from Chaetoperus eggs. J. Biophys. Biochem. Cytol. 6: 353-360.

Merriam, R.W. 1961a. On the fine structure and composition of the nuclear envelope. J. Biophys. Biochem. Cytol. 11: 559-570.

Merriam, R.W. 1961b. Nuclear envelope structure during cell division in Chaetopterus eggs. Exp. Cell Res. 22: 93-107.

Merriam, R.W. 1962. Some dynamic aspects of the nuclear envelope. J. Cell Biol. 12: 79-90.

Miller, O.L. 1962a. Cited by J. H. Taylor, 1963.

Miller, O.L. 1962b. Studies on the ultra structure and metabolism of nucleoli in amphibian oocytes. Proc. Int. Congr. Electron Micro. 5: NN8.

Miller, O.L. 1964a. Fine structure of lampbrush chromosomes in Triturus oocytes. J. Cell Biol. 23: 109 (Abstract).

Miller, O.L. 1964b. Extra chromosomal nucleolar DNA in amphibian oocytes. J. Cell Biol. 23: 60 (Abstract).

Miller, O.L. 1965a. Fine structure of lampbrush chromosomes. Nat. Cancer Inst. Monogr. 18: 79-99.

Miller, O.L. 1965b. Membranous components in amphibian oocytes (Triturus). J. Cell Biol. 27: 66 (Abstract).

Miller, O.L. 1966. Structure and composition of peripheral nucleoli of salamander oocytes. Nat. Cancer Inst. Monogr. 23: 53-66.

Miller, O.L. and B.R. Beatty. 1969. Visualization of nucleolar genes. Science 164: 955-957.

Miller, R.A. 1938. Spermatogenesis in sex reversed female and in normal male of the domestic fowl. Gallus domesticus. Anat. Rec. 70: 155-189.

Mitchison, J.M. 1966. Some functions of the nucleus. Int. Rev. Cytol. 19: 97-110.

Monne, L. 1935. Proc. Exper. Biol. Med. 32: 1197. Cited by C.M. Feldherr and C.V. Harding, 1964.

Monne, L. 1942. Arkiv. Zool. 34B No. 2. Cited by Gall, 1964.

Monneron, A. 1966. Utilization de pronase en cytochemie ultra structurale. J. Microscopie 5: 583-596.

Montgomery, T.H. 1898. Comparative cytological studies with special regards to the morphology of the nucleolus. J. Morph. 15: 266-560.

Morato, M.J. 1966. Ultrastructure of nucleolus in cat oocytes and its evolution during the mitotic prophase. C.R.S. Soc. Biol. 159: 1633-1635.

Moses, M.J. and J.R. Coleman. 1964. Structural patterns and functional organisation of chromosomes. Symp. Devel. Growth. 23: 11-49.

Naora, H., M. Izawa, V.G. Allfrey and A.E. Mirsky. 1962. Some observations on differences in composition between the nucleus and cytoplasm of the frog oocyte. Proc. Nat. Acad. Sci. U.S.A. 48: 853-859.

Nebel, B.R. and E.M. Coulon. 1962. The fine structure of chromosome in pigeon spermatocytes. Chromosoma 13: 272-291.

Nebel, B.R. and T. Hacket. 1962. Lampbrush fine structure of vertebrate male chromosomes in meiotic prophase. Naturwissenschaften 48: 655.

Newcomer, E.H. 1957. The mitotic chromosomes of the domestic fowl. J. Hered. 48: 227-262.

Newcomer, E.H. 1959. The meiotic chromosomes of the fowl. Cytologia 24: 403-410.

Newcomer, E.H. and J.W.A. Brant. 1954. Spermatogenesis in the domestic fowl. J. Hered. 45: 79-84.

Norrevang, A. 1968. Electron microscopic morphology of oogenesis. Int. Rev. Cytol. 23: 113-186.

Odor, D.L. 1960. Electron microscopic studies on ovarian oocytes and unfertilized tupal ova in the rat. J. Biophys. Biochem. Cytol. 7: 567-574.

Oguma, K. 1938. Studies on sauropsid chromosomes. v. The karyotypes of the quail and the duck, different from those reported by previous authors. Annot. Zool. Jap. 17: 612-622.

Ohno, S. 1961. Sex chromosomes and microchromosomes of Gallus domesticus. Chromosoma 11: 484-498.

Ohno, S. 1965. Personal communication.

Ohno, S., L.C. Christian and C. Stenius. 1962. Nucleolus-organizing chromosomes of Gallus domesticus. Exp. Cell Res. 27: 612-614.

Ohno, S., C. Stenius, L.C. Christian, W. Becak, and M.L. Becak. 1964. Chromosomal uniformity in the avian subclass carinatae. Chromosoma 15: 280-288.

Olsen, M.W. 1942. Maturation, fertilization and early cleavage in the hen's egg. J. Morph. 70: 513-533.

Ormsbee, R.S., I. Cornman and R.E. Berger. 1947. Effect of podophyllin on tumor cells in tissue culture. Proc. Soc. Exp. Biol. 66: 586-590.

Owen, J.J.T. 1965. Karyotype studies on Gallus domesticus. Chromosoma 16: 601-608.

Peacock, W.J. 1965. Chromosome replication. Nat. Cancer Inst. Mongr. 18: 101-123.

Peacocke, A.R. and R.B. Drysdale. 1965. The Molecular Basis of Heredity. Butterworth, London.

Perry, R.P. 1962. The cellular sites of synthesis of ribosomal and 4 S RNA. Proc. Nat. Acad. Sci. U.S.A. 48: 2179-2180.

Perry, R.P. 1966. Nucleolus: Structure and function. Science 153: 214-216.

Perry, R.P. 1967. The nucleolus and the synthesis of ribosomes. Prog. Nucleic Acid Res. Mol. Biol. 6: 219-257.

Pollister, A.W., M.E. Gettner and R. Ward. 1954. Nucleocytoplasmic interchange in oocytes. Science 120: 789.

Poole, H.K. 1963. Observation on the chromosomes of turkey-chicken hybrids. J. Hered. 54: 101-102.

Popoff, W.W. 1933. Ueber die Zahl der Chromosomen und ueber die Heterochromosomen des Haushuhnes. Z. Mikr.-Anat. Forsch. 17: 341-346.

Raven, C.H.R. 1961. Oogenesis: The Storage of Developmental Information. Macmillan, New York.

Ray-Choudhuri, R., T. Sharma and S.P. Ray-Choudhury. 1969. A comparative study of chromosomes of birds. Chromosoma 26: 148-168.

Rebhun, L.I. 1956. Electron microscopy of basophylic structure of some invertebrate oocytes. J. Biophys. Biochem. Cytol. 2: 93-104.

Ris, H. 1945. The structure of meiotic chromosomes in the grasshopper and its bearing on the nature of "chromomeres" and lampbrush chromosomes. Bio. Bull. 89: 242-257.

Ris, H. 1955. The submicroscopic structure of chromosomes, pp. 121-134. Symposium on Fine Structure of Cells (Leiden). Interscience Publishers, New York.

Ris, H. 1956. A study of chromosomes with electron microscope. J. Biophys. Biochem. (Suppl). 2: 385-392.

Ris. H. 1966. Fine structure of chromosomes. Proc. Roy. Soc., B. 164: 245-257.

Ris, H. and B.L. Chandler. 1963. The ultrastructure of genetic systems in Prokaryotes and Eukaryotes. Cold Spring Harb. Symp. Quant. Biol. 28: 1-8.

Ritossa, F.M., and S. Spiegelman. 1965. Localization of DNA complementary to ribosomal RNA in the nucleolus organizer region of Drosophila melanogaster. Proc. Nat. Acad. Sci. U.S.A. 53: 737-774.

Romanoff, A.L. 1960. The Avian Embryo. Macmillan, New York.

Rothfels, K., M. Aspeden and M. Mollison. 1963. The W-chromosome of the budgerigar, Melopsittacus undulatus. Chromosoma 14: 459-467.

Ruckert, J. 1892. Zur Entwicklungsgeschichte des Ovarialeis bei Selachiern. Anat. Anz. 7: 107-158.

Scharrer, B. and S. Wurzelmann. 1967. Ultra structure studies of nucleolar activity in oocytes of lung fish. Anat. Rec. 157: 316-317.

Schmid, W. 1962. DNA replication patterns of heterochromosomes in Gallus domesticus. Cytogenetics 1: 344-352.

Sharma, G.P., R. Prasad and A. Krishan. 1962. Chromosome number in the domestic fowl. Res. Bull. (Punjab University, India) 13: 245-251.

Sharma, A.K. and A. Sharma. 1965. Chromosome Technique. Butterworth, London.

Shinke, N. 1937. Cytologia, Fuyii Jubilee Volumen 449-463. Cited by C.M. Feldherr and C.V. Harding, 1964.

Shiwago, P.J. 1924. The chromosome complexes in the somatic cells of male and female of the domestic chicken. Science 60: 45-46.

Shoffner, R.N. 1965. Current knowledge about chromosomes in the domestic fowl. World's Poult. Sci. J. 21: 157-165.

Shoffner, R.N. and A. Krishan. 1965. The karyotype of Gallus domesticus with evidences for a W-chromosome. Genetics 52: 474-475.

Shoffner, R.N., A. Krishan, G.F. Haiden, R.K. Bammi and J.S. Otis. 1967. Avian chromosome methodology. Poult. Sci. 46: 333-344.

Sichel, G. 1966. On the ultra structure of the pores in the nuclear envelope. Cellule 66: 95-106.

Sluiter, J.W. 1939. Die Cytologie des Huehnereies waehrend der ersten Phasen der Oogenese. Protoplasma 34: 303-430.

Sokolow, N.N., G.G. Tiniakow and J.E. Trofimow. 1936. On the morphology of chromosomes in the Gallinaceas. Cytologia 7: 466-489.

Sokolow, N.N. and I.E. Trofimow. 1933. Individualitact der Chromosomen und Geschlechtsbestimmung beim Haushuhn, Gallus domesticus. Z. Ind. Abst. und Vererbungsl. 65: 327-352.

Sonnenbrodt, R. 1908. Die Wachstumsperiode der Oocyte des Huhnes. Arch. mikrosk. Anat. Entw-mech. 72: 415-480.

Srivastava, M.D.L. 1965. Cytoplasmic inclusions in oogenetics. Int. Rev. Cytol. 18: 73-93.

Srivastava, M.D.L. and A.N. Bhatnagar. 1962. Lampbrush chromosomes in oocytes of Rana cyanophylyctis. Cytologia. 27: 60-71.

Steven, A.R. 1966. The machinery of exchange across the nuclear envelope, pp. 190-211. The Control of Nuclear Activity. Edited by L. Goldstein. Prentice Hall, New York.

Stevens, B.J. and H. Swift. 1966. RNA transport from nucleus to cytoplasm in chironamas salivary gland. J. Cell Biol. 31: 55-77.

Suzuki, K. 1930. On the chromosomes of domestic fowl. Zool. Mag. (Tokyo). 42: 358.

Swanson, C.P. 1957. Cytology and Cytogenetics. Prentice Hall, New York.

Swift, H. 1956. The fine structure of annulate lamellae. J. Biophys. Biochem. Cytol. (Suppl). 2: 415-418.

Szollosi, D.J. 1965. Extension of nucleoli from pronuclei of the rat. J. Cell Biol. 25: 545-562.

Taylor, J.H. 1963. The replication and organization of DNA in chromosome, pp. 65-111. Molecular Genetics. Academic Press, New York.

Tomlin, S.G. and H.G. Callan. 1951. Preliminary account of electron microscopic study of chromosomes from newt oocytes. Quart. J. Micr. Sci. 92: 221-224.

Trosko, J.E. and S. Wolff. 1965. Strandedness of Vicia faba chromosomes as revealed by enzyme digestion studies. J. Cell Biol. 26: 125-135.

Unger, H. 1936. Beittrag zur Chromosomen Forschung der Voegel. Z. Zellforsch. 25: 476-500.

Vincent, W.S. 1964. The nucleolus. Proc. Int. Cong. Genet. 11: 343-358.

Wagenar, E.B. and D. Mazia. 1969. Personal communication.

Waldeyer, W. 1870. Eirstock and Ei Leipzig: Engelman. Cited by G. C. Hughes, 1963.

Watson, M.L. 1955. The nuclear envelope. Its structure and relation to cytoplasmic membranes. J. Biophys. Biochem. Cytol. 1: 257-270.

Watson, M.L. 1959. Further observations on the nuclear envelope of animal cell. J. Biophys. Biochem. Cytol. 6: 147-156.

White, M.J.D. 1932. The chromosomes of the domestic chicken. J. Genet. 26: 345-349.

Wilson, C.B. and J. H. Morrison. 1966. Cytology, p. 95. Reinhold Publ. Corporation, Chapman & Hall, London.

Wischnitzer, S. 1957. Study of lateral loop-chromosomes of amphibian oocyte by phase contrast microscopy. Amer. J. Anat. 101: 135-168.

Wischnitzer, S. 1958. An electron microscope study of the nuclear envelope of amphibian oocyte. J. Ultrastruct. Res. 1: 201-272.

Wischnitzer, S. 1960. The ultrastructure of nucleus and nucleo-cytoplasmic relations. Int. Rev. Cytol. 10: 137-162.

Wischnitzer, S. 1961. Amphibian oocyte nucleus. Nucleus. (Calcutta). 4: 177-198.

Wischnitzer, S. 1963. Vesicle formation from the nuclear envelope in amphibian oocytes. Chromosoma 13: 600-608.

Wittex, M. 1952. La vitellogénese chez les Amphibiens. Arch. Biol. (Paris). 63: 133-197.

Wolfe, J. 1967. Structural aspects of amitosis: A light and electron microscope study of the isolated macronuclei of Paramecium aurelia and Tetrahymena Pyriformis. Chromosoma 23: 59-79.

Wolfe, S.L. 1965. The fine structure of isolated chromosomes. J. Ultrastruct. Res. 12: 104-112.

Wolfe, S.L. and J. Bernard. 1965. The organisation and ultrastructure of male meiotic chromosomes in Oncopeltus faciatus. Chromosoma 17: 85-103.

Wolfe, S.L. and G.M. Hewitt. 1966. The strandedness of meiotic chromosomes from Onocopeltus. J. Cell Biol. 31: 31-42.

Wolfe, S.L. and P.G. Martin. 1968. The ultrastructure and strandedness of chromosomes from two species of vicia. Exp. Cell Res. 50: 140-158.

Yamashina, Y. 1943. Studies on sterility in hybrid birds. IV. Cytological researches on hybrids in the family Phassianidae. J. Fac. Sci. Tokyo Univ. 4: 307-386.

Yamashina, Y. 1944. Karyotype studies in birds. 1. Comparative morphology of chromosomes in seventeen races of domestic fowls. Cytologia (Tokyo). 13: 270-296.

Yasuzumi, G., A. Odate and Y. Ota. 1951. Fine structure of salivary chromosomes. Cytologia (Tokyo). 16: 233-242.

APPENDIX I

COMPARISON OF COLCEMID (CIBA) AND PODOPHYLLIN AS MITOTIC ARRESTORS

Literature Review

Although several mitotic arrestors have been described (Sharma and Sharma 1965, Gelfant 1963), colchicine or its derivative, Colcemid (CIBA), is the mitotic arrestor most commonly used in chromosome studies of animals and plants. Its action on dividing cells in healthy and cancer tissues has been reported by Levine (1951) and a detailed account outlining a specific mode of action of colchicine has been given by Borisy and Taylor (1967a, 1967b). During the preliminary phase of this experiment Colcemid was not available and therefore it was necessary to determine whether another amitotic agent could be used.

King and Sullivan (1946) demonstrated the similarity of colchicine and podophyllin in the treatment of condylomata acuminata. Podophyllin was used by Kaplan (1942) for reducing veneral warts. Also in India, warts and other skin growths have been treated by being rubbed or plastered with crude podophyllin powder. Because in the treatment of warts, podophyllin possesses properties similar to colchicine it was considered that podophyllin may be used as another amitotic agent for the domestic fowl.

Podophyllin is a resin derived from the roots and rhizomes of May-apple (Podophyllum peltatum) and Indian-apple (Podophyllum emodi) which contains podophyllic acid, podophylotoxin, piero-podophyllin, and other compounds (Cornman and Cornman 1951). The

effect of podophyllin as a mitotic inhibitor on tumour cells has been investigated (Ormsbee et al. 1947, Sullivan and King 1947, Belkin 1947, Leiter et al. 1950, and Makino and Tanaka 1953).

Although most investigations have been concerned with the histopathological changes of tumor tissue cells or the decrease in mitosis after exposure of podophyllin, Cornman and Cornman (1951) reported the effects of podophyllin and colchicine on normally dividing cells were essentially the same, that is, both destroy the structural organization of the mitotic apparatus. Makino and Tanaka (1953) used podophyllin for cytological studies on cell division and growth of ascites tumors to produce mitotic plates. They demonstrated that the chromosomes were condensed into irregular shapes, and cessation in the growth of tumour was due to death of the cell which had not been able to proceed beyond metaphase.

Materials and Methods

Day old and one, two, three and four week old White Rock chicks were obtained from the University of Alberta Poultry Farm.

Solutions (0.05%) of Colcemid and podophyllin were prepared. In day old and one week old chicks 0.05 ml per 30 grams of body weight of the desired solution was injected intra-peritonially. In two, three, and four week chicks, for each gram of body weight 0.08 ml of the desired solution was injected into the wing vein. After 30-40 min a primary pin feather was plucked from the wing.

Squash preparations were prepared as outlined in Chapter I.

The slides were stained with acetic orcein, observed, and the number of cells under mitosis recorded.

Results and Discussion

The mitotic index observed is shown in Table 1A. The mitotic index for podophyllin was greater than for Colcemid. This would suggest that podophyllin is a more effective mitotic arrestor than Colcemid. Typical chromosome spreads, shown in Fig. 1, 2, and 4 for podophyllin and Fig. 3 for Colcemid, are quite similar and indicate that podophyllin has not affected the chromosomal morphology differently than Colcemid. Therefore podophyllin may replace Colcemid as a mitotic arrestor in karyogram studies or obtaining well spread chromosomes with little or no detrimental effect on chromosomal morphology.

Observation indicated that podophyllin did not cause the clumping or breakage of chromosomes as claimed by Makino and Tanaka (1953). Not only were microchromosomes of the mitotic plate not broken but neither were the chromosomes in prophase (Fig. 4). Because any amitotic agent when used in more than the required dosage causes clumping or fragmentation of chromosomes, the amount used by Makino and Tanaka may have been greater than required.

The manner in which podophyllin acts on the dividing cells of the fowl is not known. However, Gelfant (1963) using podophyllin and other drugs for studying the inhibition of cell division, in vitro, of mouse ear epidermis demonstrated that podophyllin resulted in more mitotic inhibition than colchicine.

The results also indicate decreased mitotic activity of the feather pulp cells as the chicks advanced in age. As no growth

Table 1A. Mitotic index of growing feather follicle cells after injection of amitotic agents

Age	Podophyllin			Colcemid		
	Mitotic index %	No. of chicks	Cells counted	Mitotic index %	No. of chicks	Cells counted
Day old	17.8	2	2,000	17.1	2	2,367
One week	13.6	3	3,020	11.0	1	1,116
Two weeks	12.4	2	1,969	8.3	1	1,101
Three weeks	10.9	2	2,411	-	-	-
Four weeks	8.6	2	2,188	6.0	2	2,196

promoting factor was introduced into the system, the drugs used in this experiment have arrested the dividing cells which were normally occurring in the feather pulp cells. Bloom and Buss (1968) observed a similar trend in mitotic rates in embryonic tissue of White Leghorn fowls.

APPENDIX II

EQUIPMENT FOR ISOLATION OF LAMPBRUSH CHROMOSOMES

A. Isolation Chamber

The isolation chamber was prepared by drilling a one quarter inch hole in a standard microscope slide. Using paraffin wax the lower orifice of the slide was sealed with either another slide or a coverslip. This slide, sealed on one side, will be referred to as a well slide. Initially Permount was used for sealing the bottom of the well. However, it proved to be unsatisfactory because of refraction. The upper orifice of the well slide was sealed after the isolated material, nucleoli or lampbrush chromosomes, was spread in the well. The slide sealed on both sides will be referred to as the isolation chamber.

B. Preparation of the Ultrafine Tungsten Needle

For breaking the nuclear wall and removing the nucleoli and lampbrush chromosomes it was necessary to have an ultrafine needle which was dark grey rather than transparent as glass or shining as metal. A piece of tungsten wire 1 inch long and 0.005-0.01 inch diameter was sealed into a handle of pyrex glass tubing. The tip of the wire was flamed in oxygen gas just above the inner bright cone. The wire had a white glow which disappeared abruptly at the tip. Immediate removal from the flame gave a very fine point to the needle (wire). A bath containing an equal quantity of sodium nitrate and sodium sulphite was contained in a porcelain crucible. The

mixture was fused by means of a blast burner. The needle was very quickly dipped in and withdrawn from the molten chemicals.

A very high temperature of the molten mass, indicated by a yellow glow, and a very low temperature, indicated by formation of crust above and around and the fused mixture, were considered undesirable. The fineness and darkness of the needle depended on how fast and how frequently it was immersed and withdrawn. The needle was then washed in boiling water after which it was ready for usage (Hamburger 1960).

C. Formaldehyde Vapour Chamber

A formaldehyde vapour chamber was constructed by placing a coarse wire screen in a petri dish containing 40% formaldehyde. The petri dish and the course screen were kept in a covered jar.

D. Moisture Chamber

A filter paper soaked with distilled water was placed on the bottom of a petri dish. Two matchsticks were placed in such a manner that the slide, with the inspection chamber in the middle, rested evenly on both sticks. The dish was covered to prevent evaporation.

E. Blowing Pipette

A 2 mm dia glass tubing was drawn so as to have a neck of 2 inches with an internal wedge about its neck and a diameter of 0.5-7 mm at the mouth. The other end of the pipette was connected to a flexible rubber tubing which was placed in the mouth. This pipette was used during cleaning or transfer of the nucleus.