HISTONE PROTEIN TRANSITION IN *DROSOPHILA MELANOGASTER*

II. Changes During Early Embryonic Development

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ABSTRACT

Employing cytochemical methods it was found that during the early embryonic development of *Drosophila melanogaster* the nuclei contain in sequence two kinds of chromosomal proteins. The cleavage nuclei (as also the pronuclei), until shortly before the blastoderm stage, contain an atypical (or juvenile) histone, stainable with bromophenol blue but not with alkaline fast green. The typical fast green–positive histone appears at the close of the period of the synchronized cleavage mitoses, just before blastulation, when nucleoli are first produced. The amount of DNA of the cleavage nuclei, as determined cytophotometrically, is nearly constant; therefore, the DNA moiety of the nucleohistone complex seems to remain unaffected by the protein shift during embryonic development. The implications of the protein shift in relation to the histone control of gene expression are discussed.

INTRODUCTION

The cytochemical studies reported here have shown that changes occur in the composition of nuclear histones during embryonic development in *Drosophila melanogaster*. These changes afford new evidence for evaluating the alleged role of histones in controlling the course of differentiation.

Considerable attention has been given in recent years to the possibility that histones regulate gene activity (38). The concept of regulation stems from the early speculation of the Stedmans (44) that histones, by virtue of their association with deoxyribonucleic acid (DNA) in the nucleus, serve in the regulation of genic expression during embryonic development. In addition to the obvious significance of histone localization, two lines of evidence have been adduced in support of the possibility that histones control gene action: (*a*) the inhibition of transcription *in vitro* by polycations (4, 5, 13, 14, 22, 24); and (*b*) the variations in histone composition that occur in association with developmental changes (1, 6–9, 11, 12, 18, 19, 29). Such variations could conceivably alter the configurational pattern of the DNA-protein complex in a manner that would modify the specificity of action of the DNA component (9). Further light is shed on this possibility by the present study on *D. melanogaster*, in which the variations occurring during embryonic development can be defined precisely with respect to specific cleavage mitoses and the shifting patterns of organization of the embryo.

MATERIALS AND METHODS

The procedure of Sonnenblick (43) was employed (with slight modification) for the mass collection of eggs of the Swedish-b stock of *D. melanogaster*. The

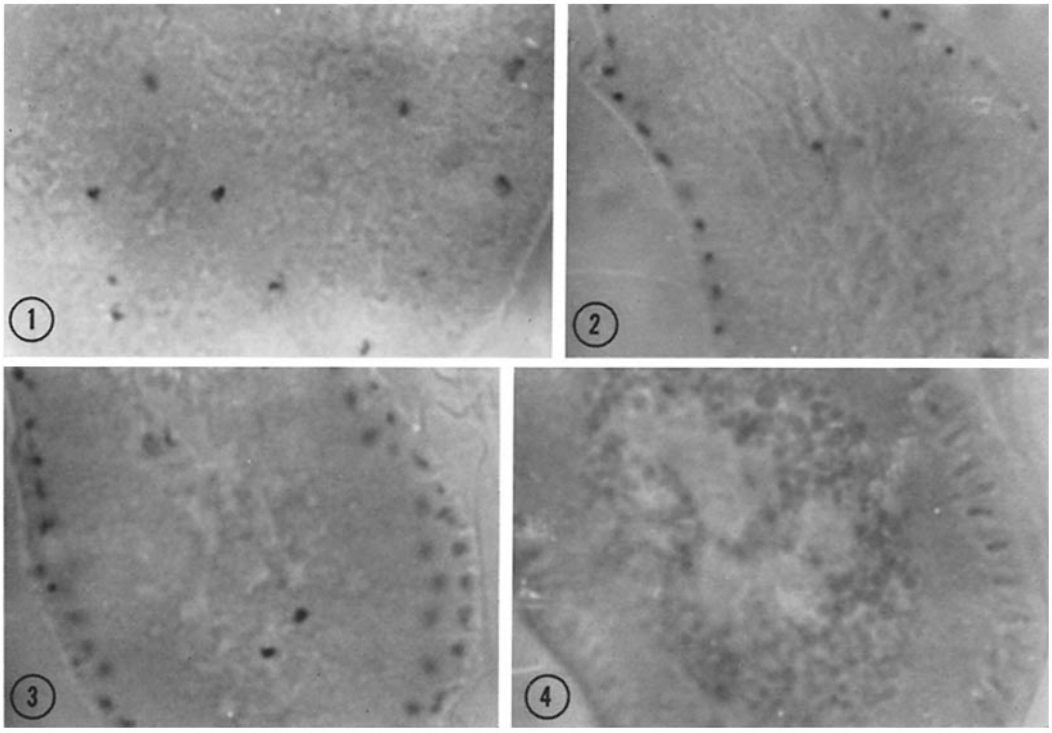

FIGURE 1 Transverse section of an egg, showing synchronous division of nuclei in the protoplasmic islands. Nuclei are distributed at random throughout the interior of the egg. Full metaphase figures during seventh cleavage. × 750.

FIGURE 2 Longitudinal section of part of an egg. Nuclei are at the late anaphase stage and are arranged in a single layer at the surface during tenth cleavage. × 750.

FIGURE 3 Horizontal (and partly tangential) section of an egg showing the accentuated peripheral disposition of nuclei at late metaphase stage of the eleventh cleavage. A few nuclei fail to reach the surface. The initiation of plasma membrane formation foreshadows the termination of the syncytial condition of the embryo. × 750.

FIGURE 4 A portion of the wall of the early blastoderm. Nuclei have lost their original spherical shape and appear elongated and enlarged. The furrows demarcating individual cells at the periphery are seen. Nucleoli (not seen) appear at this stage. × 750.

eggs were maintained for known periods of time in an incubator regulated to operate at 29 ± 0.5°C. The early ontogenic stages of the embryo were determined from the table of development worked out by Rabinowitz (40).

For the study of nuclear proteins, eggs of known age were punctured in 10 per cent neutral formalin, using tungsten needles that had been "sharpened" in fused sodium nitrite. The eggs were allowed to remain in the fixative for 6 to 12 hours at room temperature (although the length of fixation is of minor importance, since formalin has the advantage of preserving the material without marked deformation even when used for long periods of time). After fixation, the eggs were washed thoroughly in running water. They were then dehydrated and embedded in paraffin for sectioning at 12 μ. Quantitative studies of DNA were made on 12 μ sections of tissue fixed in ethanol-glacial acetic acid (3:1) for 1 to 3 hours and embedded in paraffin.

Two staining procedures were used primarily for the demonstration of basic proteins in selected stages of sectioned material, namely, the trichloroacetic acid (TCA)-fast green method of Alfert and Geschwind (3) and the picric acid-bromophenol blue method of Bloch and Hew (11, 12). Whereas the

former is allegedly specific for histones that are predominantly lysine-rich, the latter is designed to show, separately or collectively, TCA-soluble histones or protamines as well as histones stainable by the Alfert-Geschwind technic.

Further information about the histones was sought by application of the procedures of Van Slyke (45) and Monné and Slautterback (34), as outlined by Bloch and Hew (12). Both deamination with nitrous acid and acetylation under prescribed conditions are believed to affect primarily the amino groups of lysine rather than the guanidine groups of arginine (21, 36).

connected to an RCA ultrasensitive microammeter through a power supply and control unit made by the Farrand Optical Company, Inc., New York.

OBSERVATIONS

The sequence of events occurring in the egg of *Drosophila* soon after fertilization may be briefly summarized as follows. The first maturation division is in progress at the time of fertilization, and shortly thereafter the second division is completed to furnish the prospective female pronucleus. Concurrently with the organization of the female

TABLE I

Summarized Account of the Histone Transition during Spermatogenesis, Fertilization, and Early Development of Drosophila melanogaster

Nuclear types	Fast green	Fast green after deamination	Eosin Y	Eosin Y after acetylation	Bromophenol blue	Bromophenol blue after acetylation	Nature of basic protein
Spermatogonium thru early spermatozoa	+	−	+	−	+	−	Lysine-rich histone (adult type)
Maturing and fully mature spermatozoa	+	+	+	+	+	+	Arginine-rich histone (spermatid type)
Male pronucleus	−	−	−	−	+	−	Lysine-rich histone (cleavage type)
Zygote nucleus	−	−	−	−	+	−	Lysine-rich histone (cleavage type)
Syncytial nucleus (embryonic mitoses 1 to 9)	−	−	−	−	+	−	Lysine-rich histone (cleavage type)
Syncytial nucleus (embryonic mitoses 10 to 11)	+	−	+	−	+	−	Lysine-rich histone (adult type)
Blastoderm and Gastrula nuclei	+	−	+	−	+	−	Lysine-rich histone (adult type)

The Feulgen procedure as outlined by Leuchtenberger (30) was adopted for the quantitative study of chromosomal DNA. The stained sections, after dehydration and clearing, were mounted in Shillaber's Oil (R. P. Cargille Laboratories, Inc., New York) having a refractive index of 1.540, which seemed very close to that of the sectioned material. Quantitative estimations of DNA were made on metaphase chromosomes by the two wave length method of Patau (37), using a microspectrophotometer patterned after the one described by Pollister and Moses (39). This is essentially a photomicrographic apparatus in which a Bausch and Lomb 250 mm grating monochromator is used in conjunction with a Bausch and Lomb compound microscope; the light device consists of an RCA 1P21 phototube,

pronucleus, the condensed chromatin of the spermatozoon undergoes structural and chemical alteration as the sperm head is transformed into the male pronucleus. When the male and female pronuclei have approached each other and lie side by side (in the interior of the egg), they appear as two very weakly staining spherical vesicles without marked differentiation of their chromosomal materials. However, in preparation for the first cleavage mitosis, the pronuclei undergo further reorganization, for their chromosomes gradually condense and then pass as two separate groups onto the first cleavage spindle (exhibiting thereby the phenomenon of gonomery), as pointed out by

Huettner (25). A pair of diploid nuclei is formed at the end of the first cleavage; thereafter the nuclei undergo a series of mitoses in rapid succession, without cell formation, to create the syncytial type of egg. Descriptions of the exact number of cleavage mitoses that occur in *D. melanogaster* have varied with different authors, but according to our observations the synkaryon of the fertilized egg in this species passes through eleven cleavage mitoses before it gives rise to the multicellular blastoderm, an observation consonant with that of Rabinowitz (40). The nuclear divisions are synchronous, and the division products, with the exception of those set aside to form the yolk nuclei and those of the pole cells, increase by powers of two.

content would be expected to be identical with that of the sperm. The mature spermatozoon of *Drosophila* strongly binds fast green even after deamination (Table I) and contains an arginine-rich histone that is not a protamine (17, 19, 23). Failure of the pronuclei to stain with the Alfert-Geschwind method may thus reflect either a loss or transformation of chromosomal histones.

The nuclei of the cleavage mitoses likewise do not stain with alkaline fast green (nor with alkaline eosin), until the tenth cleavage mitosis (Table I). This is true of chromosomes at the condensed stages as well as the interphases, although for technical convenience and facility in comparing nuclei at different stages of development our studies were limited exclusively to the metaphase

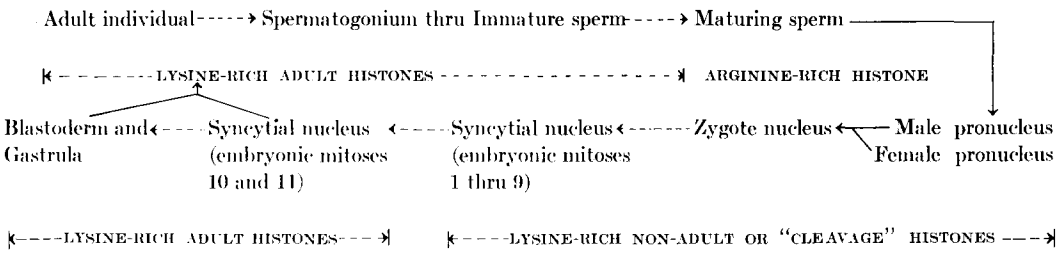

FIGURE 5 Scheme given above shows that the transition from a typical lysine-rich histone to an arginine-rich type occurs during sperm maturation rather than at the spermatid stage, and that the latter is not replaced by protamine. The arginine histone is replaced at fertilization, however, by an atypical or "cleavage" type of histone which persists until the close of the cleavage mitoses when it is replaced by the regular type of histone.

The onset of the tenth cleavage mitosis is marked by migration of the nuclei, from a heretofore random distribution throughout the interior of the egg (Fig. 1), to the surface, where they become arranged in an essentially single, cortical layer (Fig. 2). This disposition is further emphasized at the eleventh or last cleavage mitosis (Fig. 3); at the end of this division the nuclei enlarge considerably and develop nucleoli, becoming transformed thereby into the blastoderm type (Fig. 4).

On attempting to stain formalin-fixed, sectioned material with the TCA–fast green procedure of Alfert and Geschwind (3) for nuclear histones, it is observed that the chromosomal elements of the pronuclei do not stain. With respect to the pronucleus, which is derived from the spermatozoon involved in fertilization, its basic protein chromosomes. The pronuclei and cleavage nuclei react positively, although weakly, to the bromophenol blue test (Table I), which is stated to be specific for nuclear basic proteins (11, 12); the loss of stainability with this method after acetylation or deamination rules out the possibility that the protein is a protamine or arginine-rich histone. The stainable complex is believed on the basis of Bloch's criteria, to be a histone, the so called cleavage histone (or preferably a juvenile histone, since the cleaving egg of *Drosophila* before blastoderm formation is a syncytium and not a multicellular structure), as distinguishable from the fast green–positive adult histone.

The return of the chromosomes to a condition in which they stain with alkaline fast green occurs, according to our evidence, at the tenth cleavage division (about 100 minutes after oviposition at

29°C), shortly before blastoderm formation, indicating a change in the protein to a more typical histone (Table I). In the early embryonic development of *Drosophila*, we find, therefore, a pattern of histone transition from the cleavage to the adult type, similar to that postulated by Bloch and Hew (12) from a developmental study of *Helix aspersa*. However, the switch-over in the developmental protein cycle is operative in *D. melanogaster* even

TABLE II

Amount of DNA Per Diploid Set of Chromosomes During First to Eleventh Cleavages and in Neuroblasts of Third-Instar Larvae

Cleavage mitoses	No metaphase nuclei	Sample size	Mean amount DNA (arbitrary units)	t value
1	1	9	18.78	1.66
2	2	12	19.28	0.68
3	4	12	19.71	0.35
4	8	10	19.57	0.61
5	16	23	20.63	0.18
6	32	17	20.72	0.11
7	64	23	21.44	0.47
8	128	12	21.26	0.12
9	256	27	21.08	0.14
10	512	27	21.70	0.66
11	1024	33	21.95	1.02
Neuroblast metaphase nuclei		26	21.43	1.39

Over-all mean = 20.97

Statistical comparison of each individual sample (stage) with the over-all mean showed no significant difference at the 5 per cent level using Student's t tests.

before the blastoderm stage of development and not at the presumptive gastrula stage of ontogeny as noted for the snail (Fig. 5).

As already stated, the nuclei enlarge considerably just before blastoderm formation and appear to be relatively more active physiologically, as evidenced by the presence of nucleoli (usually 1 per cell). These nuclei stain feebly with the Alfert-Geschwind technic, whereas at the time of gastrulation they have again shrunk to the normal size and are brilliantly stained. The reduced staining intensity of blastoderm nuclei seems to be due largely to a dilution effect following nuclear enlargement. This assumption seems justified by the fact that the nucleoli, which presently appear, are clearly fast green–positive in otherwise very weakly staining nuclei. The ground cytoplasm as well as the contained yolk also react positively to the alkaline fast green reaction, as will be reported elsewhere.

With a view to determining whether the synthesis of DNA is affected by a shift in the nature of basic proteins during early development, since both of these nuclear components have been reported to increase in amount concurrently during mitosis (10) and to be related obligatorily in their synthesis (20), measurements of the DNA were undertaken by the usual cytophotometric method. The results indicate that there is no significant change in the amount of DNA per diploid set of chromosomes from the first to the eleventh cleavage mitoses (Table II). The amount also corresponds closely, as would be anticipated, with that present in the diploid nucleus of the larval neuroblast. These observations are in harmony with the theory of constancy in the amount of DNA per chromosome set.

DISCUSSION

The observations recorded above of successive replacements of nuclear basic proteins in the developmental cycle of *Drosophila melanogaster* parallel in many ways those of Bloch and Hew (12) which deal with the replacement of basic proteins during the life cycle of *Helix aspersa*, apparently conforming to this pattern: lysine-rich histone (adult) → arginine-rich histone (spermatid) → protamine (sperm) → lysine-rich "cleavage histone" (pregastrula) → lysine-rich adult histone. There is one striking difference, however. In the mature spermatozoa of the fruitfly, unlike those of the snail, the nuclear proteins are not extractable by hot TCA and they bind fast green even after deamination or acetylation. These observations therefore suggest that the spermatozoa contain an arginine-rich histone rather than a protamine (19, 23, 29). The functional advantage inherent in the acquisition of protamine by the spermatozoa of many species is seemingly satisfied by the presence in the sperm of *Drosophila* of an arginine-rich histone. Under such conditions the necessity of a further shift to a more arginine-rich protein or to a protamine would be obviated.

With respect to its reaction to fast green staining, the DNA-associated protein of the mature

spermatozoon of the fruitfly loses on entering the ovum the capacity of stainability with fast green that is typical of histones. It is conceivable that this response could merely mean that a conversion to protamine is delayed until fertilization or shortly thereafter. If this were so, the male pronucleus should stain with alkaline eosin as used after picric acid hydrolysis (12); but it does not, according to our observations. In the mouse, the loss of stainability of the male pronucleus has been ascribed to masking of histones by other proteins (2), since the Sakaguchi test reveals a concentration of protein-bound arginine that is high enough to delimit it clearly from the surrounding cytoplasm. But the arginine-containing protein in the male pronucleus of the fertilized egg of *Drosophila*, as determined by the Sakaguchi test, is no greater in concentration than that of the surrounding cytoplasm (the nuclei are often indiscernible), so that the lack of fast green staining is, in our opinion, mainly due to a dilution effect caused by enlargement of the sperm head in the process of its transformation into the pronucleus.

The shift from a juvenile to an adult type of histone in *Drosophila* parallels that postulated by Bloch and Hew (12) for *Helix*. Early cleavage nuclei of *Drosophila*, which fail to stain with fast green, behave in the same manner as the pregastrula nuclei of *Helix*. Our observations are based on the condensed chromosomes of the metaphase nuclei and it is reasonable to suppose, therefore, that the inability of these nuclei to stain with fast green does not reflect either a dilution or a masking of proteins but, on the contrary, the presence of a different type of basic protein, which colors with bromophenol blue although not with alkaline fast green.

The nuclei of the cleaving egg of *Drosophila* are devoid of nucleoli. The absence of nucleoli until the blastoderm stage suggests that the nuclei of the syncytial egg, although capable of replicating chromosomal material, are incapable of synthesizing RNA and hence unable to support protein synthesis—presumably by rendering the ribosomes metabolically inactive, since they do not receive messenger RNA's (15). While, however, it is not established that the nucleolus acts specifically in funnelling the genetic information carried by the messenger RNA's to the sites of protein synthesis in the cytoplasm, the assumption that nuclei lacking nucleoli may inhibit the production of messenger RNA's, thereby affecting the metabolic activity of the cytoplasmic ribosomes, may have validity (41, 42). At least the chromosomes of the fruitfly during the cleavage mitoses (as far as we can judge from Unna's methyl green–pyronin reaction, when used before and after deoxyribonuclease treatment) do not contain appreciable amounts of RNA. In any event it seems improbable that the genes would play a major role in the production of specific proteins during the syncytial phase of embryogenesis.

The very nature of the histone complex of the cleavage chromosomes, as predicted from the work of Allfrey *et al.* (5), would nevertheless endow the gene loci with a potential activity and make them vulnerable to cytoplasmic influence. As we know, the change to the characteristic lysine-rich histone occurs in nuclei at the close of the period of synchronized cleavage mitoses. This transition is followed by nucleolus formation coincident with the onset of blastoderm formation. The initiation of such events may be construed as indicative of nuclear intervention in the synthesis of RNA and, in turn, of protein synthesis during development, and is consonant with the view that the first critical period of gene activation does not become operative until the beginning of gastrulation in a developing embryo (15, 35).

In conclusion it may be stated that in our appraisal of the functional aspects of histones in the cell economy we have introduced an element of speculation. Although the possibility cannot be excluded that histones are not always involved in determination of genetic specificities, a plausible functional relationship between histones and DNA's in the control of gene action seems to exist in some cases. The functional basis of cytodifferentiation, as has recently been re-emphasized by Waddington (46), is the interaction between the genes of the zygote and the cytoplasm soon after fertilization. In the mosaic type of egg of the Diptera, the nucleocytoplasmic relationship may be assumed to involve a complex series of interactions under the control of the genetic system itself. It is only within recent years that specific concepts have emerged about controlling elements in the genotype (16, 26–28, 31–33), but our knowledge of the method of coordination of gene activities still remains meagre. It is hoped that specific information about the time of histone transition, such as is given above, will help to

clarify the speculative aspects of current interpretations.

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REFERENCES

1. ALFERT, M., Chemical differentiation of nuclear proteins during spermatogenesis in the Salmon, *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 109.
2. ALFERT, M., Cytochemische Untersuchungen an basischen Kernproteinen während der Gametanbildung, Befruchtung, und Entwicklung, Ges. physiol. Chem. Colloq., 1958, **9,** 73.
3. ALFERT, M., and GESCHWIND, I. I., A selective staining method for the basic proteins of cell nuclei, *Proc. Nat. Acad. Sc.*, 1953, **39,** 991.
4. ALLFREY, V. G., and MIRSKY, A. E., Evidence for the complete DNA-dependence of RNA synthesis in isolated thymus nuclei, *Proc. Nat. Acad. Sc.*, 1962, **48,** 1590.
5. ALLFREY, V. G., LITTAU, V. C., and MIRSKY, A. E., On the role of histones in regulating ribonucleic acid synthesis in the cell nucleus, *Proc. Nat. Acad. Sc.*, 1963, **49,** 415.
6. BLOCH, D. P., DNA-histone association as a basis for alternative hereditary gene states, *Science*, 1962, **138,** 985.
7. BLOCH, D. P., On the derivation of histone specificity, *Proc. Nat. Acad. Sc.*, 1962, **48,** 324.
8. BLOCH, D. P., Histone synthesis in non-replicating chromosomes, *J. Histochem. and Cytochem.*, 1962, **10,** 137.
9. BLOCH, D. P., Genetic implications of histone behavior, *J. Cell. and Comp. Physiol.*, 1963, **62,** suppl. 1, 87.
10. BLOCH, D. P., and GODMAN, G. C., A microphotometric study of the synthesis of desoxyribonucleic acid and nuclear histone, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 17.
11. BLOCH, D. P., and HEW, H. Y. C., Schedule of spermatogenesis in the pulmonate snail *Helix aspersa*, with special reference to histone transition, *J. Biophysic. and Biochem. Cytol.*, 1960, **7,** 515.
12. BLOCH, D. P., and HEW, H. Y. C., Changes in nuclear histones during fertilization, and early embryonic development in the pulmonate snail *Helix aspersa*, *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 69.
13. BONNER, J., and HUANG, R. C. C., Properties of chromosomal nucleohistone, *J. Mol. Biol.*, 1963, **6,** 169.
14. BONNER, J., HUANG, R. C. C., and GILDEN, R. V., Chromosomally directed protein synthesis, *Proc. Nat. Acad. Sc.*, 1963, **50,** 895.
15. BRACHET, J., Nucleic acids in development, *J. Cell. and Comp. Physiol.*, 1962, **60,** suppl. 1, 1.
16. BRINK, R. A., Phase change in higher plants and somatic cell heredity, *Quart. Rev. Biol.*, 1962, **37,** 1.
17. DAS, C. C., KAUFMANN, B. P., and GAY, H., Histone-protein transition during spermiogenesis, fertilization, and early embryonic development in *Drosophila melanogaster*, *Abst. Am. Soc. Cell. Biol.*, 1962, 39.
18. DAS, C. C., KAUFMANN, B. P., and GAY, H., Chromosomal changes during cleavage mitoses in *Drosophila*, *Carnegie Inst. Year Book*, 1962, **61,** 469.
19. DAS, C. C., KAUFMANN, B. P., and GAY, H., Histone-protein transition in *Drosophila melanogaster*. I. Changes during spermatogenesis., *Exp. Cell Research*, 1964, **35,** 507.
20. DE, D. N., Autoradiographic studies of nucleoprotein metabolism during the division cycle, *Nucleus*, 1961, **4,** 1.
21. DIETCH, A. D., Microspectrophotometric study of the binding of the anionic dye, naphthyl yellow S, by tissue sections and by purified proteins, *Lab. Inv.*, 1955, **4,** 324.
22. FRENSTER, J. H., ALLFREY, V. G., and MIRSKY, A. E., Repressed and active chromatin isolated from interphase lymphocytes, *Proc. Nat. Acad. Sc.*, 1963, **50,** 1026.
23. GAY, H., Histone transition during *Drosophila* spermiogenesis, *Carnegie Inst. Year Book*, 1963, **62,** 505.
24. HUANG, R. C. C., and BONNER, J., Histone as a suppressor of chromosomal RNA synthesis, *Proc. Nat. Acad. Sc.*, 1962, **48,** 1216.
25. HUETTNER, A. F., Maturation and fertilization in *Drosophila melanogaster*, *J. Morphol.*, 1924, **39,** 249.
26. JACOB, F., and MONOD, J., Genetic regulatory mechanisms in the synthesis of proteins, *J. Mol. Biol.*, 1961, **3,** 318.
27. JACOB, F., and MONOD, J., Elements of regulatory circuits in bacteria, *in* UNESCO Symposium on Biological Organization, (R. J. C.

Harris, editor), New York, Academic Press, Inc., 1963, 1.

28. JACOB, F., and MONOD, J., Genetic repression, allosteric inhibition, and cellular differentiation, *in* Cytodifferentiation and Macromolecular Synthesis, (M. Locke, editor), New York, Academic Press, Inc., 1963, 30.
29. KAUFMANN, B. P., and GAY, H., Cytological evaluation of differential radiosensitivity in spermatogenous cells of *Drosophila*, *in* Repair from Genetic Radiation Damage and Differential Radiosensitivity in Germ Cells, (F. H. Sobels, editor), London, Pergamon Press Ltd., 1963, 398.
30. LEUCHTENBERGER, C., *in* General Cytochemical Methods, (J. F. Danielli, editor), New York, Academic Press, Inc., 1958, **1,** 220.
31. MCCLINTOCK, B., Chromosome organization and genetic expression, *Cold Spring Harbor Symp. Quant. Biol.*, 1951, **16,** 13.
32. MCCLINTOCK, B., Some parallels between gene control systems in maize and in bacteria, *Am. Naturalist*, 1961, **95,** 265.
33. MCCLINTOCK, B., Further studies of gene-control systems in maize, *Carnegie Inst. Year Book*, 1963, **62,** 486.
34. MONNÉ, L., and SLAUTTERBACK, D. B., The disappearance of protoplasmic acidophilia upon deamination, *Arkiv. Zool.*, 1950, **1,** 455.
35. MOORE, B. C., Histone and differentiation, *Proc. Nat. Acad. Sc.*, 1963, **50,** 1018.
36. OLCOTT, H. S., and FRAENKEL-CONRAT, H., Specific group reagents for proteins, *Chem. Rev.*, 1947, **41,** 151.
37. PATAU, K., Absorption microphotometry of irregular shaped objects, *Chromosoma*, 1952, **5,** 341.
38. PHILLIPS, D. M. P., Histones, *Progr. Biophysics and Biophysic. Chem.* 1962, **12,** 211.
39. POLLISTER, A. W., and MOSES, M. J., A simplified apparatus for photometric analysis and photomicrography, *J. Gen. Physiol.*, 1949, **32,** 567.
40. RABINÓWITZ, M., Studies on the cytology and early embryology of the egg of *Drosophila melanogaster*, *J. Morphol.*, 1941, **69,** 1.
41. SIRLIN, J. L., The nucleolus, *Progr. Biophysics and Biophysic. Chem.*, 1962, **12,** 25.
42. SIRLIN, J. L., The intracellular transfer of genetic information, *Internat. Rev. Cytol.*, 1963, **15,** 35.
43. SONNENBLICK, B. P., The early embryology of *Drosophila melanogaster*, *in* Biology of Drosophila, (M. Demerec, editor), New York, John Wiley & Sons, Inc., 1950, 62.
44. STEDMAN, E., and STEDMAN, E., Cell specificity of histones, *Nature*, 1950, **166,** 780.
45. VAN SLYKE, D. D., A method for quantitative determination of aliphatic amino groups, *J. Biol. Chem.*, 1911, **9,** 185.
46. WADDINGTON, C. H., Specificity of ultrastructure in developing cells and its genetic control, *J. Cell. and Comp. Physiol.*, 1962, **60,** suppl. 1., 93.