than the short arm (*20*), contained 11 percent of the grains in the cell, and the short arm contained only 2 percent (*21*).

The data in Table 1 indicate that the long, labeled arm of the X chromosome was selectively sensitized to irradiation by the incorporation of BUdR. In the long arm, the frequency of aberrations was five times higher in the BUdR-treated cells than in the thymidine-treated cells. In contrast, no radiosensitization was apparent in the short, unlabeled arm of the X chromosome (*22*).

The radiosensitization associated with BUdR incorporation is *not* caused by 5-bromouracil increasing the absorption of energy from ionizing radiation (calculated to be an increase of less than 1 percent) (*8*). Also, the amount of tritium incorporated in the cells (22 percent more for H^3-thymidine than for H^3BUdR) did not introduce any apparent complications. In control cells labeled with H^3-thymidine (50 μg/ml) at a specific activity of either 0.2 μc/μg or 0.05 μc/μg there were 0.21 and 0.27 aberration per cell, respectively. For the higher specific activity, an average of 17 H^3 disintegrations would have occurred in the cells prior to fixation. This is equivalent to about 5 rads of x-rays and has been shown to produce a negligible amount of chromosomal damage in the Chinese hamster cell (*18*).

These results clearly illustrate that the late replicating arm of the X chromosome both incorporated about five times as much BUdR and sustained about five times as much chromosomal damage as the early replicating arm. In the irradiated cells treated with thymidine, however, the two arms sustained about the same amount of chromosomal damage. Therefore, where selective incorporation of the BUdR occurred, there was also selective radiosensitization. This correlation is consistent with previous studies (*3, 5, 6, 23*) showing that an increase in the incorporation of thymine analogs causes an increase in the radiosensitization of the cell. Furthermore, this correlation supports the hypothesis that BUdR sensitization of chromosomes to ionizing radiation is specific for the regions of incorporation and is caused by the replacement of thymine with 5-bromouracil. As discussed before (*3, 6, 10, 23, 24*), it is possible that sensitization is caused by 5-bromouracil in the DNA either interfering with repair of lesions induced near the site of incorporation or reducing the amount of energy required to damage the DNA.

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13. Tritiated thymidine (1.9 c/mmole) obtained from Schwarz BioResearch, and H^3BUdR (0.588 c/mmole) obtained from Nuclear-Chicago Corp.
14. Irradiation conditions: 250 kv(peak), 15 ma; filtered with 0.5 mm of Cu and 1.0 mm of Al, giving a half-value layer of 1.26 mm of Cu.
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21. From the tritium radioactivity of the cells, measured by liquid-scintillation counting (*3, 18*), it was calculated that the amount of thymine replaced by 5-bromouracil was 2.8 percent in the metaphase cells and 11 percent in the long arm of the X chromosome.
22. For thymidine treatment only, thymidine treatment plus 200 r, BUdR treatment only, and BUdR treatment plus 200 r there were, respectively, 0.21, 1.9, 0.44, and 3.4 aberrations per cell and 0.05, 0.13, 0.05, and 0.22 aberration per Y chromosome.
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3 March 1966

Biosynthesis and Composition of Histones in Novikoff Hepatoma Nuclei and Nucleoli

Abstract. *Histones were prepared from isolated nuclei and nucleoli of the Novikoff ascitic hepatoma at several time points after the injection of L-lysine uniformly labeled with C^{14} into tumor-bearing rats. Amino acid analysis and starch-gel electrophoresis failed to reveal any differences between the nuclear and nucleolar histones, although both fractions were more acidic in composition than calf thymus histones. However, the nucleolar histones were a metabolically distinct fraction, and their rate of synthesis was approximately twice that of the total nuclear histones.*

The importance of the nucleolus for synthesis and methylation of ribonucleic acid (RNA) has been established by many authors (*1*). The possibility that nucleoli may have a significant role in protein synthesis has also been investigated. Birnstiel and Hyde (*2*) and Birnstiel and Flamm (*3*) demonstrated rapid synthesis of proteins in pea seedling nucleoli; among other nucleolar proteins, histones became labeled to a significant extent. Rees, Rowland, and Varcoe (*4*) have noted incorporation of labeled amino acids into proteins of a nuclear fraction containing nucleoli. They concluded that the lipid-rich material, probably the heterochromatin associated with the nucleoli, was the active site for the incorporation of amino acids by rat liver nuclei. However, Busch and his co-workers were unable to provide evidence for synthesis of histones in nucleoli of mammalian tissues either in vivo or in vitro, although acidic nuclear proteins were actively labeled (*5*). Since histones seem to play an important role in regulating the biosynthesis of ribosomal RNA in nucleoli (*6*), we have attempted to determine whether the biosynthesis of histones by nucleoli is a special feature of plants or whether similar biosynthesis also occurs in mammalian cells.

Male albino rats (200 to 250 g) bear-

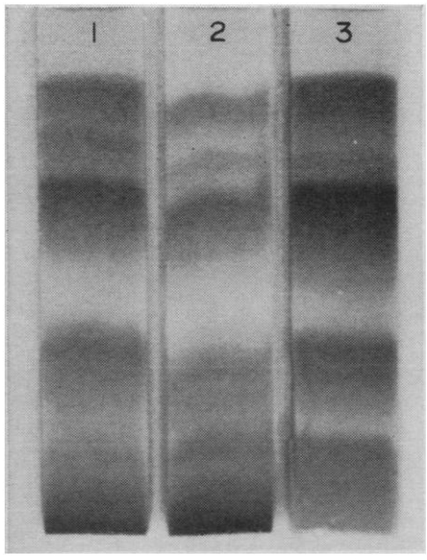

Fig. 1. Starch-gel electrophoretic patterns of histones from Novikoff ascitic hepatoma nuclei (1), nucleoli (2), and from calf thymus (3).

ing the Novikoff ascitic hepatoma were injected intraperitoneally with L-lysine uniformly labeled with C^{14} (20 μc/kg) on the 6th day after the transplantation of tumor. At various intervals after the administration of the isotope animals were killed and ascitic fluid was mixed with three volumes of ice-cold 0.25*M* sucrose and centrifuged at 1200*g* for 15 minutes in a refrigerated centrifuge. The packed cells were resuspended in approximately ten volumes of 0.25*M* sucrose and resedimented. After the centrifugation, two-thirds of the sediment was used for the isolation of nucleoli (*6*), and one-third was used for the isolation of nuclei (*7*).

Both the sedimented nuclei and nucleoli were extracted twice by homogenization in 0.14*M* NaCl containing 0.01*M* trisodium citrate and once in 0.1*M* tris-HCl buffer, *p*H 7.6. The histones were then obtained from the residue by two extractions with approximately ten volumes of 0.2*N* HCl each, and the extracts were centrifuged. The clarified extracts were pooled, dialyzed against deionized water, and lyophilized.

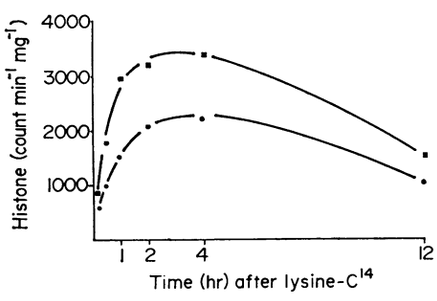

Fig. 2. The rate of incorporation of uniformly labeled L-lysine-C^{14} into nuclear (●) and nucleolar (■) histones of Novikoff ascitic hepatoma.

Isolated histones were analyzed for amino acid content and by starch-gel electrophoresis (*7*). For radioactivity determination, the histones were dissolved in distilled water to make a 1.0 percent solution; portions (1.0 mg) were pipetted onto Whatman No. 3 MM filter paper discs which were dried at 110°C for at least 12 hours. The specific activity of the samples was determined from scintillation spectrometer counts of the paper discs.

The amino acid composition and the appearance of the acid-soluble proteins from nuclei and nucleoli confirmed our findings that histones are present in mammalian nucleoli (*6*). In comparison with other mammalian histones (such as calf thymus), the Novikoff hepatoma nuclear and nucleolar histones are more acidic (Table 1); there is no significant difference between Novikoff hepatoma nuclei and nucleoli in the amino acid composition of histones. The relatively low content of lysine and alanine may indicate a decreased amount of the very lysine-rich fraction 1 in the nucleolar histones in Novikoff hepatoma (Table 1). The lower basicity of histones in Novikoff hepatoma nuclei and nucleoli as compared with calf thymus histone was not due to a contamination with cytoplasmic proteins since the isolation procedure of nucleoli practically excluded such contamination.

The acid-soluble proteins in Novikoff hepatoma nuclei and nucleoli are histones, showing the typical distribution of the three main fractions (F3, F1, and F2 in order of increasing electrophoretic mobility, Fig. 1). Both the amino acid composition (low basicity) and the starch-gel electrophoretic patterns of Novikoff hepatoma histones strongly resemble histones in embryonic tissues recently analyzed in this laboratory.

The kinetics of the incorporation of L-lysine uniformly labeled with C^{14} (Fig. 2) demonstrates that a rapid synthesis of histones occurs in nucleoli of the Novikoff hepatoma in vivo. The decrease in specific activity of the labeled histones after 3 hours is at least partially due to dilution by growth of the tumor, rather than to metabolic turnover. The data indicate that histones of the nucleoli are a metabolically distinct fraction, biosynthesized more actively than the other histones of the cell nucleus. If the histones isolated from the entire nucleus also contain the more rapidly labeled nucleolar histones, then the nucleolus seems to biosynthesize histone at a rate about twice that of the nucleus. Birnstiel and Flamm working with pea seedlings and with exponentially dividing tobacco cell cultures reported a rapid biosynthesis of histones in the nucleoli which was approximately twice the nucleoplasm value (*3*). It can be concluded that rapid biosynthesis of histones in nucleoli seems to be a feature common to both plants and animals.

Table 1. Amino acid composition of histones from Novikoff hepatoma nuclei (Nu) and nucleoli (No), compared with calf thymus histones (CTH). The amino acids are expressed as moles per 100 moles of all amino acids found. A correction (10 percent) was made for the hydrolytic losses of serine. Amide and the N^6-methyllysine were not determined.

Amino acid	Nu	No	CTH
Lysine	13.5	13.4	14.4
Histidine	1.9	1.9	1.9
Arginine	7.2	7.6	8.6
Aspartic acid	7.4	7.4	4.8
Threonine	5.2	5.1	5.6
Serine	6.9	6.7	6.0
Glutamic acid	11.4	11.5	9.0
Proline	5.1	4.8	5.0
Glycine	8.1	8.3	8.4
Alanine	10.7	10.1	13.7
Valine	5.8	5.7	5.6
Methionine	1.3	1.5	1.1
Isoleucine	3.7	3.8	4.2
Leucine	7.4	7.6	7.7
Tyrosine	2.2	2.4	2.4
Phenylalanine	2.3	2.5	1.5
Total basic	22.6	22.9	24.9
Total acidic	18.8	18.9	13.8

The rapid biosynthesis of histones in nucleoli is difficult to explain. The possibility that the site of biosynthesis of all histones is nucleolus has been suggested by Birnstiel and Flamm (*3*); the shape of the incorporation curves over the period of 12 hours (Fig. 2) does not, however, strongly indicate that in Novikoff hepatoma the nucleolus is the source of all nuclear histones.

At least a part of nucleolar histones is utilized for repression of the DNA not participating in the biosynthesis of nucleolar ribosomal RNA (*6*). The average lifetime of a protein repressor in mammalian cells is not known. However, histones, due to their lack of a rigid tertiary structure, should be easily digested by proteolytic enzymes and

thus require continuous replacement. Whether the rate of histone renewal is related to rapidity or control of RNA synthesis, or strictly coupled with the synthesis of DNA, remains still undetermined.

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Bivalve Mollusks: Fluid Dynamics of Burrowing

Abstract. *When bivalves burrow into soft substrates the foot is first extended and then dilated to obtain a firm anchorage before retraction pulls the shell downward. Pedal dilation is principally caused by adduction of the valves. The hinged shell functions as a hydraulic machine in which the strength of the adductor muscles is transferred to the distal part of the foot by means of the body fluids.*

It has long been established that the water contained in the mantle cavity of *Mya arenaria* is used as the fluid of a hydraulic system that enables either contractions of the adductor muscles to extend the siphons, or retraction of the siphons to open the valves (*1*). Past research on the locomotion of Bivalvia (*2*) suggests that burrowing movements of the foot represent the action of the pedal musculature together with the inflow of blood. Recent investigations show that, at adduction of the valves, high pressure occurs in both the hemocoel and the mantle cavity, and that such pressures are utilized in burrowing.

In my investigation, knowledge of the fluid dynamics of bivalves was considerably extended by the use of electronic recording techniques (*3*). Used were a multichannel pen recorder (*4*), isotonic and isometric myographs attached by a thread to a valve to record movement, transducers (*5*) to determine internal pressures, and cine film, sometimes synchronized with the pen recorder, for recording details of digging activity. Film was exposed both at the commencement of burrowing (with the subject on the surface of the sand) and through an aquarium tank filled with sand, when the bivalve burrowed sufficiently closely to the glass side. The pressure transducers were connected to the bivalve by a hypodermic needle (0.9-mm bore) and a short length of pressure tubing; the needle, inserted into the pedal hemocoel or mantle cavity of *Ensis* through the fourth pallial aperture of the mantle, did not obstruct burrowing until the tubing reached the surface of the sand, with at least one-half of the shell buried. In *Mya* and *Margaritifera*, short hypodermic needles were securely fastened with wax in small holes drilled in the valves (Fig. 1, *A* and *B*); specimens kept for several months with the needles attached remained very active. Movement of the valves of clams buried in the sand was recorded by attaching a very light wire electrode to each valve and passing a small a-c signal, from an impedance pneumograph, between them; small variations in current occasioned by movement of the valves were recorded (Fig. 2*c*). The operation of all transducers and the location of the hypodermic needles were checked after each experiment.

Investigation of the fluid dynamics of bivalves was preceded by observations of their burrowing behavior. Genera representative of bivalves living in sand or mud (for example, *Nucula, Glycymeris, Anodonta, Cardium, Tellina, Donax, Mercenaria, Mactra*, and *Ensis*) all show an essentially similar series of steplike digging movements. Each step represents a cycle of activity (Fig. 3) incorporating the following successive actions: extension of the foot (*i*), closure of the siphons (*ii*), adduction of the valve (*iii*), pedal dilation (*iii–iv*), contraction of pedal retractor muscles, causing movement of the shell into the sand (*iv*), followed by a short period of relaxation (*v*) when the valves reopen. Between successive cycles there occurs a static period (*vi*) of variable duration, when the foot is extended, probing more deeply into the substrate, and the shell either remains stationary or is slightly raised (Fig. 3, *L*). Pedal retraction (*iv*) generally occurs in two phases, particularly in bivalves with wide shells: posterior retraction follows anterior, so that a rocking movement is imparted to the shell, making for easier penetration (*6*).

Protraction of and probing by the foot is carried out by the intrinsic pedal

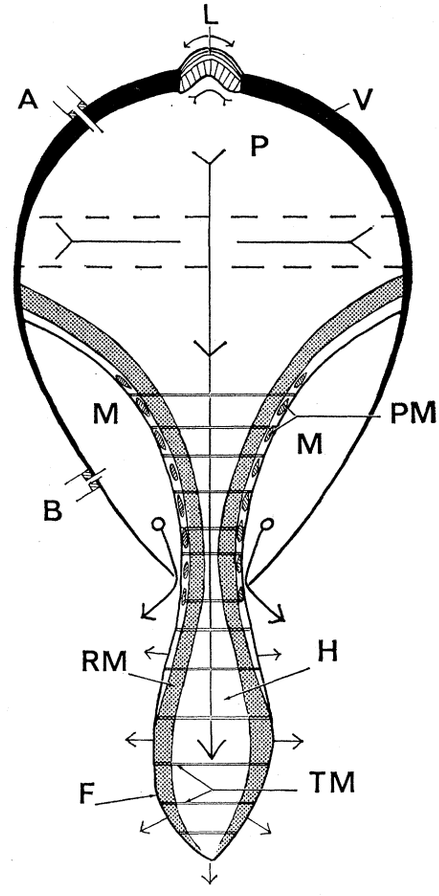

Fig. 1. Diagrammatic transverse section of a bivalve during digging (stage *iii* of Fig. 3), showing: adductor muscle contracted (broken line and >— —<) and tension (<——>) and compression (>——<) of the outer and inner layers of the ligament, respectively; ejection of water from the mantle cavity (o——→); and pressure of blood from the pericardial cavity into the foot (>——→), which causes its extension and outward dilation (——>). *A* and *B*, Location of cannulae; *F*, foot; *H*, pedal hemocoel; *L*, ligament; *M*, mantle cavity; *P*, pericardial cavity; *PM*, protractor muscle; *RM*, retractor muscle (stipple); *TM*, transverse muscle; *V*, valve.