

enzymes to have a number of active center similarities, thus suggesting that pancreatic enzymes are homologous.

Studies of porcine pancreatic ribonuclease were presented by C. H. W. Hirs and V. N. Reinhold. They have purified the enzyme but have found that it is molecularly inhomogeneous. These ribonucleases are glycoproteins and the inhomogeneity arises from a variable amount of polysaccharide around a constant protein core. All these enzymes contain 125 amino acids and have eight half-cystines. The protein core has other compositional similarities to bovine ribonuclease A.

K. Takahashi contrasted the primary structure and early mechanistic studies of ribonuclease  $T_1$  with A. There is little analogy between primary structures despite highly analogous transesterification and -2',3'-cyclic phosphate hydrolysis (of guanosine-3'-phosphodiester). The single lysine in  $T_1$  is not required for activity. Reaction with bromoacetate did inactivate the enzyme, but histidine was not alkylated; only esterification of glutamate-58 occurred. Thus, present evidence suggests that the two ribonucleases may have different catalytic mechanisms. It appeared that, despite the recent major advances in knowledge of the structure of ribonuclease A and the mechanistic implications of these findings, there is still a great deal of work to be done on the structure and mechanism of other ribonucleases and considerable informative work to be done on ribonuclease A as well.

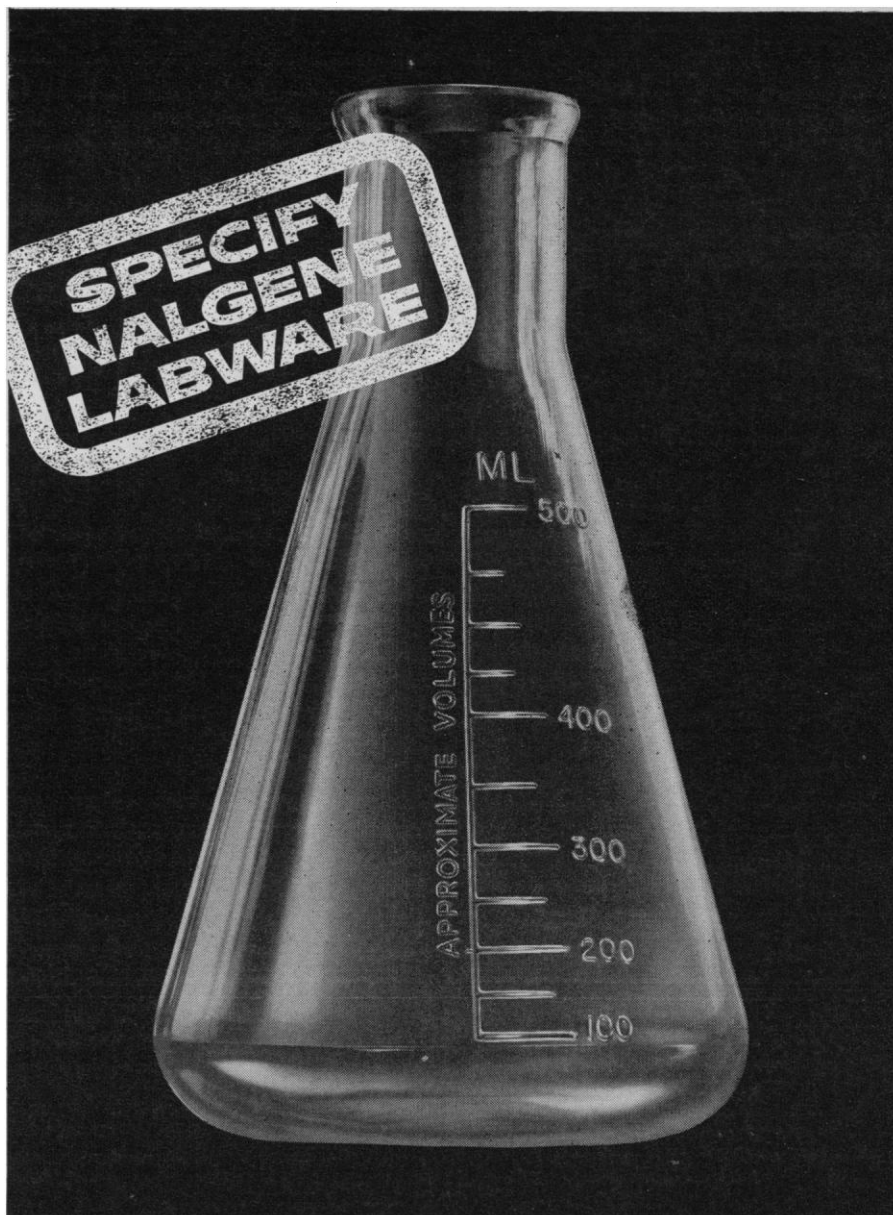
The symposium was sponsored by the Department of Biochemistry, Schools of Medicine, Dentistry and Pharmacy, State University of New York at Buffalo, and the Graduate Division of the Roswell Park Memorial Institute, Buffalo, New York.

DAVID B. STRAUS

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### Cell Synchrony

Recent advances in the synchronization of cell division was the topic at the 2nd International Conference on Cell Synchrony, held 27-29 April 1967, in Oak Ridge, Tennessee. The program was divided into five sessions: (i) Genetic studies in cell synchrony (H. O. Halvorson, University of Wisconsin, chairman); (ii) Developmental aspects of cell synchrony (D. T. Lindsay, Uni-



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versity of Georgia, chairman); (iii) Regulation and control in synchronized cells (T. W. James, University of California at Los Angeles, chairman); (iv) Biochemistry and physiology of synchronized cells (G. G. Holz, Jr., State University of New York at Syracuse, chairman); and (v) Macromolecular aspects of cell division synchrony (I. L. Cameron, State University of New York at Syracuse, chairman).

A number of contributors examined the proposal that the bacterial chromosome has a single fixed site from which replication proceeds in one direction. Since somewhat conflicting results were reported for cells artificially synchronized by different methods, a study of the replication of the bacterial chromosome during normal, steady-state growth was of particular interest. T. Nagata (Harvard University) analyzed *Escherichia coli* cells pulse-labeled with  $^3\text{H}$ -thymidine during exponential growth and then transferred to an isotopically heavy medium. He found that the density of  $^3\text{H}$ -labeled DNA shifted from that of light DNA to hybrid DNA after 0.8 to 1.1 generations and that a similar shift occurred in the second and third generations. Thus the chromo-

some has a fixed point and direction of replication for several generations during normal, steady-state growth.

Another means of studying cells during normal, steady-state growth was presented by C. E. Helmstetter (Roswell Park Memorial Institute). *Escherichia coli* cells were labeled during exponential growth, and "young" cells were collected by detachment from the surface of a membrane. His results indicated that (i) replication of the entire chromosome took 40 minutes, (ii) completion of chromosome replication preceded cell division by 20 minutes, and (iii) replication of one chromosome could begin and end in different cell-division cycles.

A somewhat different approach for studying the control of cell division in bacteria was reported by W. D. Fisher (Oak Ridge National Laboratory) on mutants of *E. coli*. A short film showed three different cell-division mutants of *E. coli* K-12 isolated by H. I. Adler (ORNL). Cytokinesis in one of these strains is blocked by x-rays, and is reversed by addition of extracts from normal cells.

Another group of participants examined ordered enzyme synthesis in

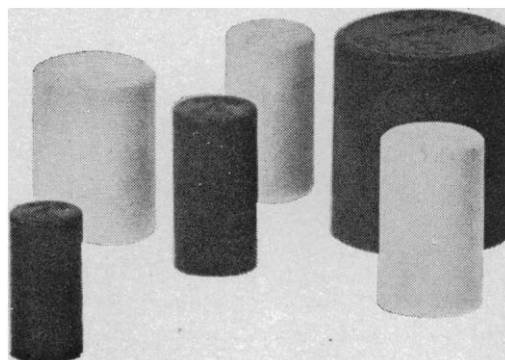
synchronized cells. P. Tauro, from Halvorson's laboratory (University of Wisconsin), reported on the analysis of ordered enzyme synthesis in synchronized budding yeast cells and showed that each structural gene has a defined period of enzyme synthesis. He brought up the interesting question of the relation of the position of structural genes to the time of their expression during the cell cycle and also of whether the yeast genome is transcribed in a sequential manner. J. M. Mitchison (University of Edinburgh) elaborated further on enzyme synthesis in synchronized fission yeast. He emphasized that while certain structural genes showed a defined period of enzyme synthesis others showed a constant rate of synthesis with a doubling at some time in the cell cycle. To account for the second pattern of synthesis, Mitchison proposed a model in which there is a delay between the replication and transcription of the yeast genome. W. D. Donachie (Hammersmith Hospital, London) presented data showing that several induced enzymes in synchronized bacteria show a doubling in rate of synthesis at a given time during the cell division cycle. The time of rate-

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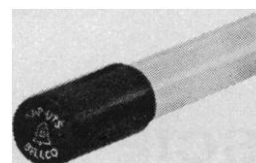
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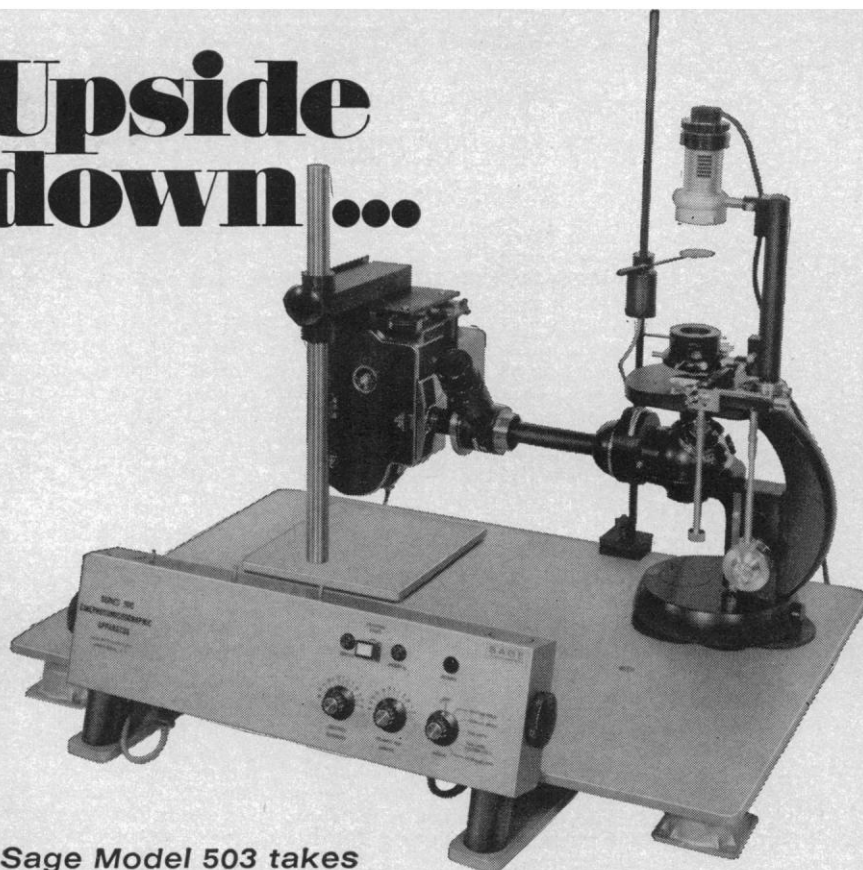
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synthesis doubling was more or less correlated with the map position of genes coding for each enzyme.

The possible role of macromolecular synthesis in regulation and control mechanisms in cells was examined extensively. H. Senger (Oregon State University) discussed light-dependent formation of nucleic acids and their relation to induction of cell division in *Chlorella*. He indicated that light of certain wavelengths influences both the formation of RNA and protein synthesis, which in turn controls the burst of cell division. R. R. Schmidt (Virginia Polytechnic Institute) reported on the enzymatic control of nucleic acid synthesis in synchronized *Chlorella*. He noted fluctuations in the levels of enzymes involved in nucleic acid synthesis. In particular, he produced enzymatic evidence that ribonucleotides in *Chlorella* are reduced to deoxyribonucleotides at the diphosphate level. A simultaneous synthesis of DNA and histone was shown in widely separated cell types: by Scharff (Albert Einstein College of Medicine) in mammalian cells (HeLa cells) and by Lindsay (University of Georgia) in the protozoan cell, *Tetrahymena*. In HeLa cells, histones were produced on specific localizable polyribosomes in the cytoplasm; the production of histones was blocked by the inhibition of DNA synthesis. Simultaneous synthesis of histone is, however, not required for DNA synthesis since G. C. Mueller (University of Wisconsin) reported that isolated HeLa cell nuclei from synchronized or logarithmically growing cells are active in DNA synthesis. A. M. Zimmerman (University of Toronto) discussed the effects of high pressure on cell division and certain cell macromolecules in *Tetrahymena*. He was unable to demonstrate any differences in protein content of dividing and nondividing cells, that is, no indication of a “division protein.” However, he found differences in ribosomal patterns between pressurized and nonpressurized cells. J. Byfield (University of California at Los Angeles) suggested that the synchronizing effect of temperature shock in *Tetrahymena* may involve messenger RNA in that temperature shifts drastically reduce the half-life of already labeled RNA.

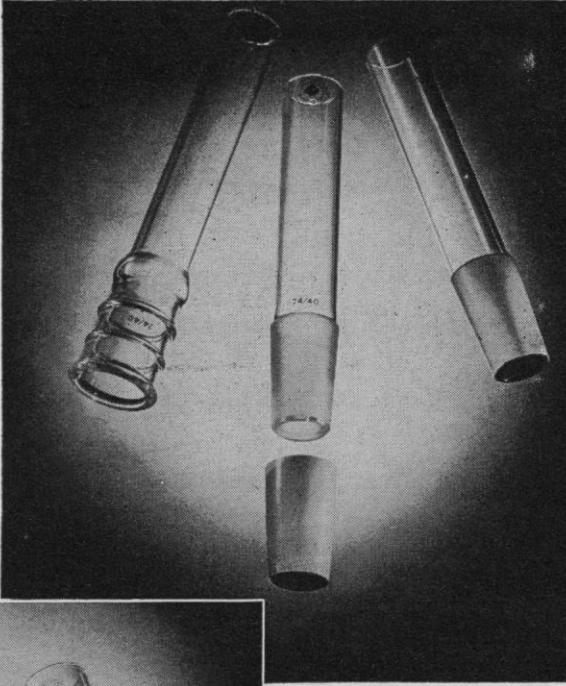
Several papers examined the biochemical events associated with cell division and differentiation. P. R. Gross (Massachusetts Institute of Technology) reported on controls of rate of protein synthesis during early development of sea urchin eggs, and gave evidence that

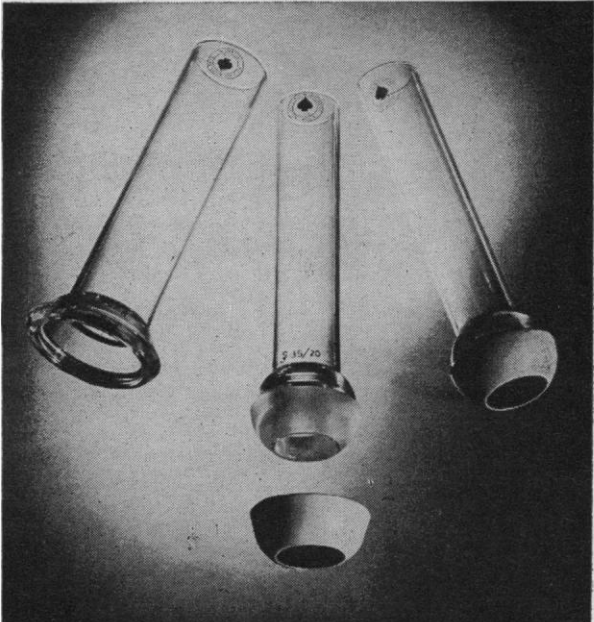
mRNA is stored in eggs and renewed mRNA synthesis occurs only after several successive cell divisions. Data presented by R. M. Iverson (University of Miami at Coral Gables) supported the idea of an increase in protein synthesis and in polysomes after fertilization in sea urchin eggs in the absence of mRNA synthesis. This was further evidence that mRNA is stored in eggs. David Epel (Hopkins Marine Station, Stanford University) reported on "early" structural and respiratory changes occurring within seconds after fertilization and "late" initiation of protein synthesis occurring about 10 minutes after fertilization. This increase in protein synthesis was attributed to an increased rate of translation of pre-existing mRNA.

The induction of synchrony in mammalian cells by various inhibitors of macromolecular synthesis received considerable attention. W. K. Sinclair (Argonne National Laboratory) demonstrated that the toxicity of various drugs is related to the stage of the cell cycle. For instance, cycloheximide (an inhibitor of protein synthesis) is more toxic to  $G_1$  cells, less toxic to cells in S phase, and even less toxic to  $G_2$  cells. D. F. Petersen (Los Alamos Scientific Laboratory) also reported on various inhibitors of biosynthesis of macromolecules in Chinese hamster cells. He was able to show specifically that cells removed from cycloheximide after varying intervals still have the capacity to divide, indicating indirectly that mRNA which directs the synthesis of proteins is relatively stable over long periods of time. Furthermore, he has shown that synthesis of essential RNA is inhibited when protein synthesis is shut off. P. N. Rao (University of Kentucky) reported on synchrony in HeLa cells by means of reversal of thymidine and colcemide blocks. While reversal of colcemide blocks could be obtained in HeLa cells, he showed that this was true only when the drug was added in low concentrations and within specific time limits after prolonged treatment.

In summarizing the conference J. M. Mitchison (University of Edinburgh) stressed the need of understanding the events in the early stages of the cell cycle which play important roles in the control of cell division. He mentioned the more recent interest in the study of the relations of biochemical events to physiological events involved in the study of cell division. In particular, he stressed the need of a better understanding of the end products of

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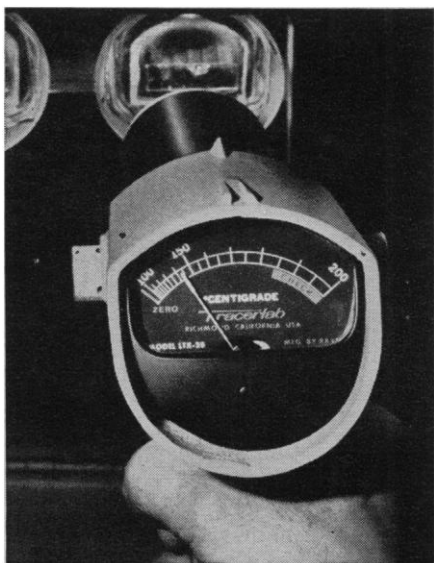
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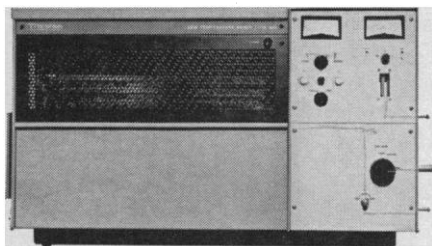
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genetic translation in cells. He further pointed out the need for new morphological or biochemical markers, or both, associated with the different metabolic states, especially during interphase. He contrasted natural synchrony with induced synchrony and pointed out some of the problems associated with the different methods of synchrony.

The conference was held under the joint sponsorship of the Biology Division of the Oak Ridge National Laboratory and Duke University. It was attended by 130 investigators from Canada, England, Scotland, Germany, Japan, Russia, and the United States and dealt with recent advances in the synchronization of cell division. Fifty-one papers were presented, and discussions were interspersed throughout the entire program.

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## Note

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## Calendar of Events

### Courses

**Hydrospace: State of the Art**, Hopatcong, N.J., 25–29 Sept. Fee: \$200. (B. Nierenberg, Oceanics Workshop, Saul Gordon Associates, Center for Professional Advancement, P.O. Box 66, Hopatcong, 07843)

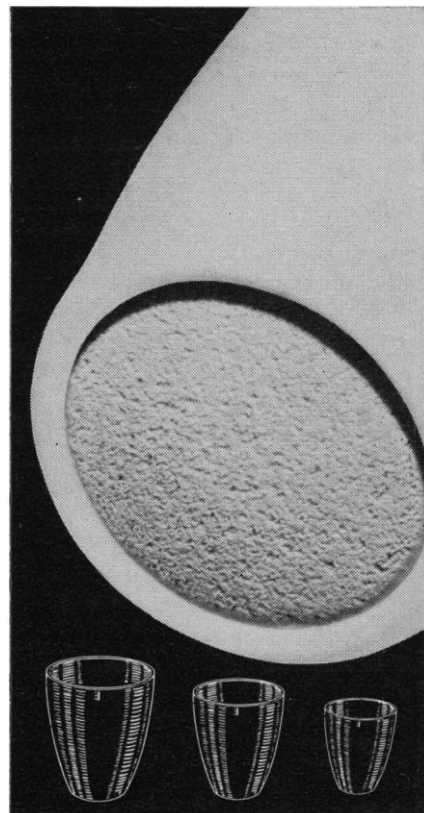
**Cardiopulmonary Problems in Children**, Chicago, Ill., 21–23 Sept. (American College of Chest Physicians, 112 E. Chestnut St., Chicago 60611)

**Cancer Chemotherapy**, Houston, Tex., 18–29 Sept. The chemistry, pharmacology, and clinical application of the antimetabolites, hormones and miscellaneous newer drugs will be reviewed. (E. Frei, 6723 Bertner Ave., Houston 77025)

**Modern Aspects of Communication Theory**, Austin, Tex., 25–29 Sept. Fee: \$175. (D. E. Griffith, College of Engineering, University of Texas, Austin 78712)

**Water Utility Management**, Urbana, Ill., 29 Oct.–2 Nov. Is intended to aid the water commissioner, manager, and supervisors to increase their management skills. (E. Lyons, 116e Illini Hall, Champaign, Ill. 61820)

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