crude venom were studied by disc electrophoresis (*11*). All fractions and the crude venom were assayed for lethal activity in mice and active fractions were further studied in certain biological assays (*12, 13*).

The lyophilized venom contained, per 100 mg, 51.4 mg of protein (smaller lots contained 43.5 to 52.6 percent protein); 2.2 mg of nonprotein nitrogen; 7.2 mg of total nitrogen; and 1.5 mg of carbohydrate. Sodium was less than 1 meq per gram of venom; potassium, 2.7 meq per gram; and calcium, 0.2 mg per gram. The venom had no proteolytic, amylase, phosphodiesterase, or L-amino acid oxidase activity; 100 mg of venom in 30 minutes (0.182 μmole per milligram per minute) hydrolyzed 547 μmole of acetylcholine.

On the paper chromatogram, a fluorescent spot with an R_F value of 0.575 and a positive Ehrlich reaction was observed. Serotonin showed fluorescence under the same conditions and had an R_F value of 0.585. Comparison of the intensity of the Ehrlich reactions indicated a serotonin concentration in the venom of approximately 4.0 μg/mg.

The elution pattern for one sample is shown in Fig. 1. The lethal activity of the venom was associated with peak 2 in all samples. This lethal component of the venom appeared to be relatively unstable. It was almost completely destroyed on lyophilization and lost its lethal effect when it was kept at room temperature. However, storage in solution at −60°C did not alter the lethal effect over a 3-month test period. It can be concentrated in a dialysis bag subjected to a draught of air at 5°C.

A second toxic group of fractions was associated with peak 4. Mice receiving injections from samples taken from this peak became ill when given doses equivalent to the lethal dose of peak 2, but recovered within 1 to 3 hours.

Disc-electrophoresis patterns of 25 individual and pooled lots of crude venom showed some variation. Samples from individual specimens within a given geographic area varied as greatly in their electrophoretic pattern as samples from area to area. From 13 to 15 bands were observed in the gels.

The intravenous LD_{50} (lethal dose, median for 50 percent of inoculated group) of the crude lyophilized venom in mice was 4.87 mg per kilogram of body weight. Even when the venom was stored at 5°C in the dark, the LD_{50} of the venom slowly changed. In 1 year, the LD_{50} of one sample fell from less than 5.0 mg to approximately 20 mg per kilogram of body weight of the test animal. In a mammalian phrenic nerve-diaphragm preparation the crude venom did not provoke any significant deleterious effect, and none of the fractions separated on Sephadex or by disc electrophoresis impaired neuromuscular transmission. The crude venom had a negligible effect on preparations of deep extensor muscles (medial) of the abdomen of the crayfish. Compared to venom from the scorpion *Centruroides sculpturatus*, approximately 100 times as much was needed to produce a complete neuromuscular block in the same length of time. Neither the crude venom nor its fractions had a significant effect on reflex discharge or on antidromic inhibition in the cat.

When the crude venom was injected into cats, it caused an immediate precipitous fall of approximately 30 percent in the systemic arterial pressure. This fall was followed by a rise, so that within 2 minutes of the injection the arterial pressure was 10 to 30 percent higher than the initial pressure. This elevated arterial pressure persisted, or in some cases increased, during the first several hours after the injection. The specific mode(s) of action for the cardiovascular changes have not yet been determined. The samples associated with peak 2 appeared to produce a similar response on a survey preparation of the mammalian cardiovascular system (*13*). However, since the quantity of each fraction was very small, a limited number of experiments were completed.

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References and Notes

1. H. L. Stahnke, personal communication.
2. J. Strassberg and F. E. Russell, *Toxicon* **1**, 41 (1962).
3. G. R. Kingsley, *J. Lab. Clin. Med.* **27**, 840 (1942).
4. O. H. Lowry *et al.*, *J. Biol. Chem.* **193**, 265 (1951).
5. F. C. Koch and T. L. McMeeken, *J. Amer. Chem. Soc.* **46**, 2066 (1924).
6. O. Folin and H. Wu, *ibid.* **41**, 367 (1920).
7. B. L. Oser, *Hawk's Physiological Chemistry* (McGraw-Hill, New York, 1965).
8. J. B. Sumner and G. F. Somers, *Chemistry and Methods of Enzymes* (Academic Press, New York, 1953).
9. J. F. Koerner and R. L. Sinsheimer, *J. Biol. Chem.* **228**, 1049 (1957).
10. D. Wellner and A. Meister, *ibid.* **235**, 2013 (1960).
11. L. Ornstein, *Ann. N.Y. Acad. Sci.* **121**, 321 (1964); B. J. Davis, *ibid.* **121**, 404 (1964).
12. F. E. Russell and T. E. Long, in *Myasthenia Gravis*, H. R. Viets, Ed. (Thomas, Springfield, Ill., 1961), p. 101; I. Parnas and F. E. Russell, in *Animal Toxins*, F. E. Russell and P. R. Saunders, Eds. (Pergamon, Oxford, 1967), p. 401; B. Renshaw, *J. Neurophysiol.* **9**, 191 (1946).
13. F. E. Russell, F. W. Buess, J. Strassberg, *Toxicon* **1**, 5 (1962).
14. Supported by NIH grant AI 03751 and NSF grant GB 3303. We thank J. Dubnoff for technical advice. F.W.B. is a trainee in the program in toxinology, University of Southern California; supported by USPHS grant GM 01700-01A1.
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Collagen Synthesis by Cells Synchronously Replicating DNA

Abstract. *Replication and the performance of a differentiated function have been considered antagonistic processes. When cells in culture are partially synchronized with 5-fluoro-2'-deoxyuridine (FUdR), the synthesis of the specialized protein (collagen) is not reduced during chromosomal replication (S period). Collagen synthesis varies with general protein synthesis through the S period.*

The idea that the production of a specialized protein was precluded in proliferating cells (*1*) was supported by the work of Holtzer, Marshall, and Fink and by Stockdale and Holtzer (*2*). These authors showed that dividing muscle cells did not bind fluorescent antibody to myosin, and that cells binding fluorescent antibody to myosin were not dividing. There are many examples, however, showing that the processes of differentiation and proliferation are not mutually exclusive. Wessels (*3*) found cells in the developing pancreas with mitotic figures and small amounts of zymogen. Mäkelä and Nossal (*4*) demonstrated that antibody synthesis was occurring in plasma cells before division had ceased. Alpha and beta crystallins were found in proliferating epithelial cells of the developing lens, although mature fibers were not seen until DNA synthesis had ceased (*5*).

Tissue culture has been a way of determining whether anything specific in cellular division precludes maximum expression of specialized function. Goldberg, Green, and Todaro (*6*) reported an aneuploid mouse line (3T6) producing about 16 mμmole of hydroxyproline in protein per 10^7 cells per day. Because hydroxyproline is

found almost exclusively in collagen, the term hydroxyproline-containing protein is assumed to be synonymous with the term collagen, as shown for 3T6 cells by Goldberg and Green (*7*). In addition to this aneuploid line from mouse embryo (*8*), a diploid line of cells established in this laboratory from rat aorta (PR105) synthesizes up to 10 percent of total protein as hydroxyproline-containing protein, and produces fibers with the characteristic ultrastructural periodicity of collagen. Many human diploid fibroblasts produce smaller amounts of collagen in culture. When radioactive proline is used as the precursor of proline in protein, and as the precursor of proline and hydroxyproline in collagen (*9*), it is possible to obtain simultaneously a measure of the rate of general protein synthesis and of the rate of collagen synthesis. Furthermore, the use of 5-fluoro-2′deoxyuridine (FUdR) to obtain synchronously dividing cell populations (*10*) permitted further study of proliferation versus differentiation. We now report simultaneous measurement of the rates of general protein and collagen synthesis throughout the burst of DNA replication after release by thymidine of FUdR block. These measurements were made to determine whether cells synthesize a specialized protein while replicating DNA and if they do, whether they synthesize that protein at a rate comparable with the rate in nonreplicating cells. The diploid and aneuploid cells studied do continue to synthesize collagen through the S phase (period of chromosomal replication), and, more interestingly, synthesize collagen at rates which fluctuate with the fluctuations in general protein synthesis.

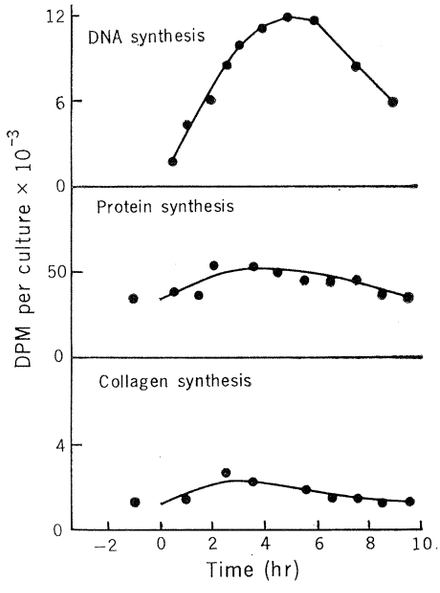

Fig. 1. DNA, protein, and collagen synthesis in partly synchronized 3T6 aneuploid mouse line. The cells were incubated for 1 hour with tritiated proline to determine protein and collagen synthesis; FudR block was released by thymidine at time 0. Each point on the graph represents one plate. In six similar experiments, collagen synthesis continued through the period of DNA synthesis and changed with general protein synthesis.

Aneuploid mouse cells (3T6) and diploid rat cells (PR105) were grown as monolayers in plastic culture dishes (60 by 15 mm) with 4 ml of Dulbecco and Vogt's medium (*11*), supplemented with 10 percent bovine calf serum and 125 μM sodium ascorbate, equilibrated with 10 percent CO_2 in a humidified desiccator and incubated at 37°C. Diploid fetal human cells (PR100) were grown in medium F10 (*12*) supplemented with 15 percent fetal bovine serum and sodium ascorbate, and equilibrated with 5 percent CO_2. The cells were partially synchronized by exposure to $4 \times 10^{-7} M$ FUdR for 16 hours, and the synchronization was reversed with $6 \times 10^{-6} M$ thymidine as described (*13*). A maximum of 90 percent of the cells begin synthesis of DNA within 10 minutes after reversal of the block. Cell division occurs 8 to 10 hours later. Synthesis of DNA was measured as incorporation of tritiated thymidine (1 μc/ml, 0.36 c/mmole, Schwarz BioResearch) into acid-precipitable material after 15 minutes. Incorporation before reversal of the block was not measured because the radioactive thymidine would have reversed the block, although Reiter and Littlefield (*14*) using radioactive hypoxanthine, as a precursor of DNA, have shown synthesis of DNA to be absent at the time of relief of FUdR block in L cells. To measure collagen and protein synthesis the medium was replaced with serum-free medium containing tritiated L-proline (1 μc/ml, 292 mc/mmole, Nuclear Chicago) and 250 μM sodium ascorbate. After 1 or 2 hours, the protein of medium and cell layer was precipitated with four volumes of cold absolute ethanol and kept at least 3 hours at 4°C (*15*). The precipitate was washed three times with cold 0.01M L-proline in absolute ethanol, and then dried, hydrolyzed in 6N HCl at 105°C for 15 hours, and finally it was assayed for total radioactivity and radioactive hydroxyproline (*16*). Samples were counted in a Packard 3000 Series Tri-

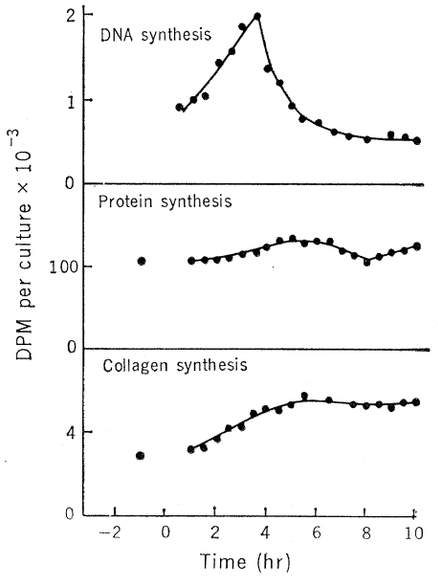

Fig. 2. DNA, protein, and collagen synthesis in partly synchronized PR105 diploid rat cells. The cells were exposed to tritiated proline for 2 hours to determine protein and collagen synthesis. A single plate was incubated every half hour. The approximation of adjacent points is a measure of the variability of the method. FUdR block was released by thymidine at time 0.

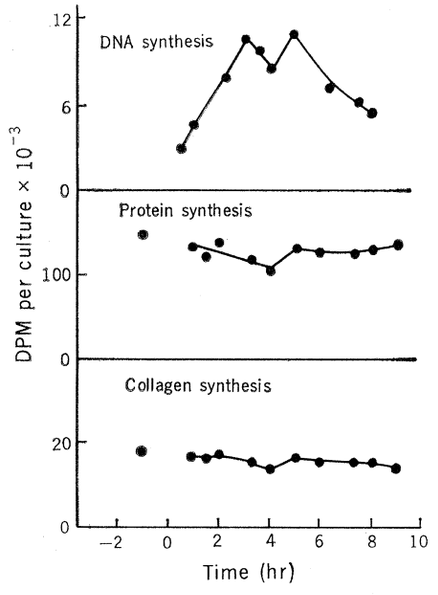

Fig. 3. DNA, protein, and collagen synthesis in partly synchronized diploid fetal human cells (PR100). The cells were incubated for 2 hours with tritiated proline to determine proline and collagen synthesis. Each point represents two plates. The variation between duplicate plates was less than 8 percent of total radioactive counts per minute for protein synthesis and less than 3 percent of total hydroxyproline counts per minute, for all but the last two time periods, when the variation was 4.8 and 6 percent respectively. The FudR block was released by thymidine at time 0.

Carb liquid-scintillation spectrometer. The efficiency of counting was determined with tritiated toluene as the internal standard. Synthesis of protein and collagen was measured just before the addition of thymidine to show that synthesis was going on during the FUdR block and to demonstrate the rate of synthesis.

In the aneuploid mouse line (Fig. 1), a burst of DNA synthesis begins immediately after the FUdR block is reversed and reaches a maximum in 4 to 6 hours. Protein synthesis is only moderately changed, increasing slightly during the first part of S from the rate prior to release of the block. Collagen synthesis also increases slightly, approximating the changes in general protein synthesis. In these aneuploid cells, therefore, specialized protein synthesis is not shut off or decreased during the S period.

To determine whether the control of synthesis of collagen in diploid cells is different from that of aneuploid cells, similar experiments were performed with diploid rat (PR105) and diploid fetal human (PR100) cell lines. Cells of PR105 make 10 percent of total protein as collagen, whereas 3.5 percent is the highest we have obtained with the aneuploid cells (3T6). In Fig. 2 a sharp burst of DNA synthesis is seen in cultures of PR105, with a peak at 3 to 4 hours after reversal of FUdR block. The rate of incorporation of proline into protein increases moderately from the level prior to addition of thymidine and the changes in collagen synthesis follow closely the changes in protein synthesis, as found for 3T6 cells. In the cultures of diploid fetal human cells (Fig. 3), synthesis of DNA was maximum 3 to 5 hours after addition of thymidine. Protein synthesis fluctuated, but showed a slight tendency to decrease during maximum DNA synthesis. Changes in collagen synthesis are again similar to those in protein synthesis.

Stollar, Buonassisi, and Sato (*17*) noted that synthesis of adrenal steroids per unit number of cultured adrenal tumor cells remained unchanged in logarithmic and stationary phases of growth. We have shown (*18*) that synthesis of collagen per unit number of cells in culture fluctuates with general protein synthesis during the logarithmic and stationary phases of growth and is more rapid during the logarithmic phase. Our results indicate that production of collagen does not cease during that portion of the cellular division cycle when DNA is replicated, but instead that it again fluctuates with changes in general protein synthesis. Moreover, aneuploid and diploid cells respond similarly. In both types of cultures the rate of transcription of collagen appears to be controlled by a mechanism that alters the rate of transcription of all proteins.

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References and Notes

1. A. Marchok and H. Herrman, *Bull. Inst. Cell. Biol.* **7**, No. 6 (1966); C. Grobstein, in *The Cell*, J. Brachet and A. F. Mirsky, Eds. (Academic, New York, 1959), vol. 4, p. 437.
2. H. Holtzer, J. Marshall, H. Fink, *J. Biophys. Biochem. Cytol.* **3**, 705 (1957); F. Stockdale and H. Holtzer, *Exp. Cell Res.* **24**, 508 (1961).
3. N. K. Wessells, *J. Cell Biol.* **20**, 415 (1964).
4. O. Mäkelä and G. Nossal, *J. Exp. Med.* **115**, 231 (1962).
5. J. Papaconstantinou, *Amer. Zool.* **4**, 279 (1964); T. Yamada and C. Takata, *J. Cell. Biol.* **27**, 114A (1965); C. Takata, J. Albright, T. Yamada, *Science* **147**, 1299 (1965).
6. H. Goldberg, H. Green, C. Todaro, *Exp. Cell Res.* **31**, 444 (1963).
7. H. Goldberg and H. Green, *J. Cell Biol.* **22**, 227 (1964).
8. Supplied by Dr. H. Green, Dept. of Pathology, New York University.
9. M. R. Stetten and R. J. Schoenheimer, *J. Biol. Chem.* **153**, 113 (1944).
10. M. A. Rich, J. L. Bolaffi, J. E. Knoll, L. Cheong, M. L. Eidinoff, *Cancer Res.* **18**, 730 (1958); J. W. Littlefield, *Exp. Cell Res.* **26**, 318 (1962); J. H. Priest, J. E. Heady, R. E. Priest, *J. Nat. Cancer Inst.* **38**, 61 (1967).
11. This medium represents an enrichment of Eagle's basal medium; it was formulated by Dr. R. Dulbecco, Salk Institute for Biological Studies, San Diego, California. The composition is found in H. Ginsburg, in *Methodologic Approaches to the Study of Leukemias*, V. Defendi, Ed. (Wistar Inst. Press, Philadelphia, 1965).
12. R. Ham, *Exp. Cell Res.* **29**, 515 (1962). This medium contains both thymidine and proline, which were omitted individually from media containing the respective radioactive element. In addition, during the time of FUdR block, the cells were incubated in medium without thymidine.
13. J. H. Priest, J. E. Heady, R. E. Priest, *J. Nat. Cancer Inst.* **38**, 61 (1967).
14. J. M. Reiter and J. W. Littlefield, *Biochem. Biophys. Acta* **80**, 562 (1964).
15. Radioactivity does not appear in hydroxyproline-containing protein until 30 minutes after addition of labeled proline, and the rate of incorporation does not parallel the rate of incorporation into the general cellular proteins until 3 hours. (R. E. Priest and L. M. Davies, *J. Cell Biol.*, in press.) The radioactive exposure time, then, is limited by the need for a short exposure to labeling material in synchrony experiments and by the length of time necessary to get sufficient radioactive incorporation into collagen.
16. K. Juva and D. W. Prockop, *Anal. Biochem.* **15**, 77 (1966). Total radioactive incorporation was assumed to represent proline in protein, and proline and hydroxyproline in collagen. Calculations were made according to H. Green and B. Goldberg, *Nature* **200**, 1097 (1963), to arrive at figures for noncollagenous protein synthesis and for collagen synthesis.
17. V. Stollar, V. Buonassisi, G. Sato, *Exp. Cell Res.* **35**, 608 (1964).
18. L. M. Davies and R. E. Priest, *Fed. Proc.* **26**, 300 (1967).
19. Supported by grant HE 08072 from USPHS. We thank H. Daniels for technical assistance.
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Isotope Separation by Carrier Diffusion

Abstract. *Nitrogen isotope fractionation in the Hoering-Moore experiment, injection of N_2 into CO_2 carrier and flow through sandstone, is due to diffusion in the gas phase rather than to surface interaction. This process, called "carrier diffusion," produces a characteristic fractionation pattern relative to a fraction coordinate, with points of zero fractionation at 16 and 84 percent and heavy isotope enrichment between these points. Carrier diffusion is an efficient enrichment process for low-abundance isotopes lighter than the abundant species and for helium and hydrogen in gas mixtures.*

Diffusional separation processes for gaseous and isotopic mixtures are of two general types: those which do not require an additional gas as a separating agent and those in which separation of the mixture occurs by diffusion through an added gas component. The first type includes the well-known process of barrier diffusion or effusion, characterized by molecular flow through a porous barrier, and the method of thermal diffusion across a temperature gradient. Processes which depend on a third component include mass diffusion (*1*), in which the mixture and a separating agent flow in opposite directions separated by a filter through which they interdiffuse, and sweep diffusion (*2*) in which a separating agent diffuses across the flowing mixture and sweeps the less-diffusible component to one side of the stream. Both mass and sweep diffusion operate with continuous flow of mixture through the separation unit and depend upon differential rates of diffusion perpendicular to the direction of flow. In this paper a one-dimensional separation process is described, in which a mixture is injected as a spike or batch into a flowing carrier gas and diffuses up and down stream from the injection point during the bulk flow. This process, called *carrier diffusion*, accounts for the nitrogen isotope separation observed in geochemical studies of gas flow in sandstones (*3*) and is applicable to unusual isotope effects observed in gas-solid chromatography (*4*) and to separation of gas mixtures in general.

In their experimental study, Hoering and Moore (*3*) injected N_2 into CO_2 flowing through a dry core of Torpedo sandstone and found that the emerging N_2 first increased and then decreased in