PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **176**, 302–308 (1984)

An *in Vitro* Model of Fibroplasia: Simultaneous Quantification of Fibroblast Proliferation, Migration, and Collagen Synthesis[1] (41875)

MARTIN F. GRAHAM,* ROBERT F. DIEGELMANN,† AND I. KELMAN COHEN†

†*Division of Plastic Surgery and* **Department of Pediatrics, Children's Medical Center, Medical College of Virginia/Virginia Commonwealth University, Richmond, Virginia 23298*

Abstract. Previous studies of fibroblast proliferation, migration, and collagen synthesis have been limited in their ability to define the interrelationship among these events in response to various inflammatory mediators. We have now defined an *in vitro* tissue culture model for the synchronous quantification of these parameters of fibroplasia. Biopsies (2 mm) of chicken flexor tendons are embedded in a fibrin matrix and exposed to various factors for 5 days in tissue culture. The availability of the fibrin matrix surrounding the tendon biopsy satisfies the need for a solid support medium for fibroblast migration. Multiple measurements of tendon fibroblast proliferation, migration into the fibrin matrix, and relative collagen synthesis are then made on these preparations. Fetal calf serum stimulated tendon fibroblast proliferation and migration in a dose responsive fashion, whereas the selective expression of collagen synthesis was decreased. Platelet lysate stimulated fibroblast proliferation at low concentration, but migration only at high concentration and was without effect on relative collagen synthesis. This model now provides a means of more clearly defining the influence of various inflammatory factors on the events of fibroplasia.

The "healing" response of tissue to injury involves complex interactions between inflammatory cells, their products, coagulation factors, hormonal and nutritional plasma elements, and the collagen-producing cells (1–4). These interactions are thought to induce the fibroblasts to move into the area of inflammation, to proliferate there, and to lay down new connective tissue protein. The precise relationship between the various inflammatory cells and their factors and the different components of the connective tissue response has been difficult to discern *in vivo* and in cell culture. *In vivo* studies are difficult to control and cell culture studies employ diverse techniques to study different phenomena. For example, *in vitro* studies employ a Boyden chamber for migration (5), a culture dish for proliferation (6), and usually a different set of defined conditions for the measurement of collagen and protein synthesis (7). In recent years, evidence has accumulated that the behavior of connective tissue cells is influenced, to a large extent, by their extracellular environment (8)—one that is highly variable under cell culture conditions. We have thus designed a tissue culture model that mimics to a limited degree the wound environment and also permits synchronous study of the events of fibroplasia within that environment.

Explants of chicken flexor tendons are embedded in a fibrin matrix and are then incubated in culture medium containing various inflammatory mediators. The fibrin matrix serves as a solid support into which the tendon fibroblasts can migrate. Tendon fibroblast migration, proliferation, and collagen synthesis are then all quantified under the same experimental conditions. Approximately 80 explants are procured from each set of tendons from a single chicken permitting quantification of replicates of each culture condition.

This paper describes the details of this *in vitro* model of fibroplasia, and how in initial studies, it has been used to discern the disparate effect of two inflammatory mediators on fibroblast migration, proliferation, and collagen synthesis.

Materials and Methods. *Experimental model.* Flexor digitorum profundus tendons were removed from the long digits of female White Leghorn chickens (2.5–3 kg) in sterile fashion, rinsed in Dulbecco's Modified Eagle's Medium (DMEM), and dissected to ensure complete removal of all sheath and other sy-

[1] Presented in part at the Federation of American Societies for Experimental Biology Annual Meeting (Fed Proc **41**:439, 1982, and Fed Proc **42**:1298, 1983).

0037-9727/84 $1.50

novial components. Plugs 2 mm in diameter were cut from the excised tendons with a dermal trephine and placed in multi-well culture plates (Costar No. 3524, Cambridge, Mass.). The tendon plugs were covered first by 10 μl thrombin solution (2 mg/ml DMEM) and then by 50 μl fibrinogen solution (KABI, Grade L, Stockholm, Sweden; 3 mg/ml). The fibrin matrix was allowed to mature at 37°C for 30 min in a humidified atmosphere after which 1 ml of DMEM supplemented with glycl-histidyl-lysine (200 ng/ml) (9), aprotinin (100 KIU/ml, Trasylol, Mobay Chemical Corp., New York), and fresh ascorbate (0.1 m*M*) were added to each well. The tripeptide, glycyl-histidyl-lysine, was used to maintain the viability of the tendon fibroblasts in the absence of exogenous serum or growth factors (9) and the aprotinin was used to ensure the stability of the fibrin matrix against breakdown by tissue proteases. This medium will be referred to as "DMEM base." After a 48-hr period of quiescence in DMEM base, various exogenous factors to be assayed were added to the medium and incubation was continued for an additional 5 days. This was the period determined in an earlier study, necessary to achieve maximal fibroblast proliferation (10). Medium supplemented with fresh ascorbate was replaced every 24 hr.

Tendon fibroblast migration. In a stimulated system (10% fetal calf serum) fibroblasts can be observed migrating into the fibrin matrix after 48 hr (10, 11). After 5 days incubation in the presence of 10% fetal calf serum, the fibroblasts have migrated out of the tendon plug into an area 5–8 mm^2 (10). Projection of the radiating fan of migrating cells onto paper then permits measurement of the area of migration using a Zeiss MOPP III automatic planimeter.

Tendon fibroblast proliferation. Incorporation of [^{125}I]iododeoxyuridine (^{125}IUDR, 2,000 Ci/mmole, New England Nuclear, Boston, Mass.) into dividing fibroblasts was used to quantitate DNA synthesis as an index of proliferation (12). After 5 days of incubation in factor-supplemented medium, the specimens were pulsed with ^{125}IUDR (1 μCi/ml) and 10^{-5} *M* 5-fluorodeoxyuridine (FUDR, Sigma, St. Louis, Mo.) for 12 hr. The duration of this pulse was determined by a time-course experiment of ^{125}IUDR incorporation into the tendon fibroblasts (Fig. 1). The FUDR enhanced ^{125}IUDR uptake by providing a specific inhibition of endogenous thymidine synthesis (13). Following the incubation, the radioactive medium was removed, the wells were rinsed three times with phosphate-buffered saline, and the contents of the wells were dissolved completely in 1 ml of 1 *N* sodium hydroxide at 37°C for 24 hr. The sodium hydroxide solution contained 2 mg of carrier DNA (Type I, calf thymus, Sigma Chemical Co., St. Louis, Mo.) to ensure complete recovery of the radioactive tendon fibroblast DNA. The alkaline treatment was used to facilitate complete tissue solubilization, hydrolyze ribonucleic acid, and destroy nucleases (14, 15). In addition, DNA is more readily precipitated by acid following alkali treatment (16).

After neutralization with 0.5 ml of 2 *N* hydrochloric acid, the samples were chilled to 4°C and protein and DNA were precipitated by the addition of 0.15 ml of 50% trichloroacetic acid (TCA) to give a final concentration of 5%. Micro stir bars (8 × 1.5 mm) were placed in each well and the culture plate was placed on a stir plate to allow adequate mixing

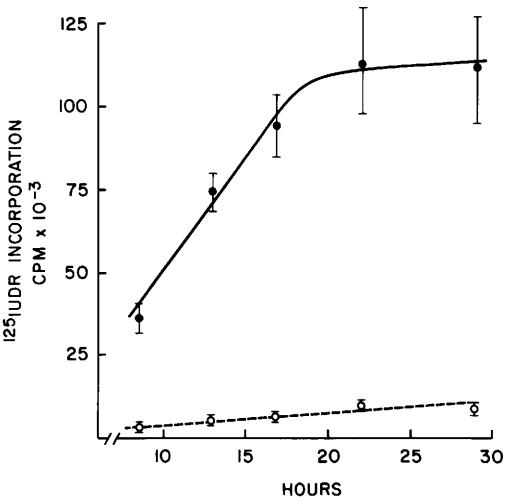

FIG. 1. Time course of ^{125}IUDR incorporation into tendon fibroblast DNA. Cultures were incubated for 5 days in the presence of 10% FCS. Solid line represents isotope incorporation for the duration of hours indicated. The dashed line represents the background activity in control specimens incubated in the presence of 25 m*M* hydroxyurea during the isotope pulsing period. The error bars represent the SEM of six replicates.

during these additions. The precipitated DNA was washed four times with 1 ml of 5% TCA in the cold to remove unincorporated isotope. To facilitate this procedure, the 24-well culture plates were centrifuged (300*g*) using microtiter centrifuge carriers (Cooke System, Dynatech Laboratories Inc., Alexandria, Va.). After the last wash, the stir bars were removed, the final precipitate was dried, and the wells were sprayed with clear plastic. The wells were then cut out of the plate with a band saw, and counted for ^{125}I radioactivity in a gamma spectrometer (12). Control specimens were preincubated for 2 hr with hydroxyurea (25 m*M*) to allow sufficient time for the DNA synthesis inhibitor to penetrate through the fibrin matrix and into the tendon fibroblasts. The use of hydroxyurea as a specific inhibitor of DNA synthesis (17) allows determination of background nonspecific binding of isotope which is then subtracted from the test samples.

IUDR is deoxythymidine with iodine substituted for a methyl group in the fifth position of the nucleotide ring. The use of ^{125}IUDR to measure DNA synthesis was chosen because with gamma radioactive counting, samples do not need to be transferred from the multi-well culture plates for analysis. In addition, the cost associated with scintillation vials and fluors needed for [^{14}C]- or [^{3}H]thymidine usage is also avoided. Iododeoxyuridine equilibrates with the thymidine pool and its incorporation into DNA can be blocked by thymidine competition (18). The use of ^{125}IUDR as a specific DNA precursor is gaining acceptance and the rate of incorporation of this isotope has been shown to be directly related to cell proliferation (19–21).

Tendon fibroblast collagen synthesis. These studies were performed on tendon cultures incubated in parallel with those used for proliferation and migration studies. Collagen synthesis was quantitated by a technique based upon the susceptibility of newly synthesized radioactive protein to be digested by purified bacterial collagenase (22). After 5 days of factor-supplemented incubation, tendon explants were incubated with the appropriate medium containing fresh ascorbate (0.1 m*M*) and 10 μCi of [^{3}H]proline (43 Ci/mmole, Schwarz/Mann, Orangeburg, N.J.). After 6 hr, the incubation was stopped by removing the radioactive medium and then rinsing the culture plate wells twice with phosphate-buffered saline (4°C). The explants, complete with fibrin matrix, were removed and placed into centrifuge tubes on ice. The wells were rinsed with 1 ml of deionized water which was added to the tissue samples. The samples were then heated to 90°C for 10 min to destroy any proteolytic enzyme activity and then frozen until time of analysis. Specimens from two wells were pooled and analyses were done in triplicate. After thawing, the radioactive protein was precipitated in the cold with TCA (5%) prior to digestion with a highly specific bacterial collagenase as previously described (22). After digestion of the collagen, the noncollagenous protein was reprecipitated with 5% TCA–0.25% tannic acid. Supernatants containing collagen-derived peptides and pellets containing the noncollagenous protein were then counted separately for radioactivity. Radioactivity released by collagenase is represented by the value in the numerator and the percentage collagen synthesized relative to total protein synthesis is calcualted according to the following formula:

$$\% \text{ collagen synthesis} = \frac{\text{cpm in collagen}}{(\text{cpm in noncollagen protein} \times 5.4) + \text{cpm in collagen}} \times 100.$$

The factor of 5.4 corrects for the enriched content of proline and hydroxyproline in collagen versus only proline in noncollagen protein (23).

Effect of various trophic factors on fibroblast proliferation, migration, and collagen synthesis. After 48 hr incubation in DMEM base, various concentrations of fetal calf serum (FCS) and platelet lysate (PL) with 5% platelet-poor plasma (PPP) were added to the incubation medium. After 5 days of additional incubation, fibroblast proliferation, migration, and collagen synthesis were determined as described above. The FCS was purchased from GIBCO Laboratories (Grand Island, N.Y.). Platelet lysate (PL) and PPP were prepared as previously described by Pledger *et al.* (24). Expired human platelets were obtained from the Medical College of Virginia blood bank and washed three times in phosphate-buffered saline. The platelet suspension was then frozen, thawed, and heated to 100°C for 5 min.

The cell debris was removed by centrifugation and the supernatant was used as the platelet lysate. The protein concentration in the lysate was estimated by 260–280-nm determinations (25).

For the preparation of PPP, freshly obtained human plasma was collected in citrated tubes and centrifuged at 3500*g*. The supernatant was collected and defibrinated by heating at 56°C for 30 min. The plasma was then centrifuged at 27,000*g* for 30 min and sterile-filtered (0.45 μm) prior to use (24).

Results. *Time course of ^{125}IUDR incorporation into tendon fibroblasts.* Incubation of tendon explants with ^{125}IUDR at intervals from 8 to 28 hr on the fifth day of incubation with 10% FCS showed a linear incorporation of isotope from 8 to 16 hr (Fig. 1). In subsequent studies, the level of background radioactivity in the hydroxyurea blanks was subtracted from the test specimens. The background value was normally less than 5% of the radioactivity incorporated into the non-hydroxyurea-treated specimens. A 12-hr pulse was, therefore, used in all subsequent experiments.

Effect of fetal calf serum on fibroblast proliferation, migration, and collagen synthesis. Both fibroblast proliferation and migration responded in a dose-related fashion to increasing concentrations of fetal calf serum in the culture medium and were maximal at the highest concentration tested (10%). In contrast, collagen synthesis, relative to total protein synthesis, was maximal at low concentrations of fetal calf serum (8–10% of total protein synthesized) and with increasing concentrations, became minimal at the 10% concentration (3.5% of total protein synthesized) (Fig. 2). When these collagen synthesis data were calculated on an absolute basis (per tendon explant), it became apparent that there was an increase in the synthesis of collagen (2.6-fold, from 0.1 to 10% fetal calf serum) but this was overshadowed by the increase in the synthesis of noncollagen protein (6.5-fold) (Table I). Therefore, the net effect resulted in a decrease in relative collagen synthesis in response to increasing concentrations of fetal calf serum.

TABLE I. INFLUENCE OF FETAL CALF SERUM ON THE SYNTHESIS OF COLLAGEN AND NONCOLLAGEN PROTEIN BY TENDON EXPLANT CULTURES

Percentage fetal calf serum	Collagen (cpm/tendon culture)[a]	Noncollagen protein (cpm/tendon culture)[a]	Relative collagen synthesis (%)
0	106 ± 26	245 ± 93	8.1
0.1	229 ± 11	490 ± 50	8.1
0.5	180 ± 27	291 ± 31	10.3
1.0	241 ± 51	467 ± 137	9.4
2.0	246 ± 37	970 ± 185	4.8
10.0	606 ± 168	3183 ± 963	3.5

[a] Data expressed as mean ± SEM; $N = 3$. Cultures were incubated for 6 hr with [^{3}H]proline (10 μCi/ml) and two tendon explants were pooled for each analysis. Collagen was specifically digested by purified bacterial collagenase and the radioactivity released into the supernatant was measured whereas noncollagen protein was reprecipitated and measured in the pellet (22). The percentage collagen synthesis was calculated after correcting for the enriched amino acid content of collagen (23).

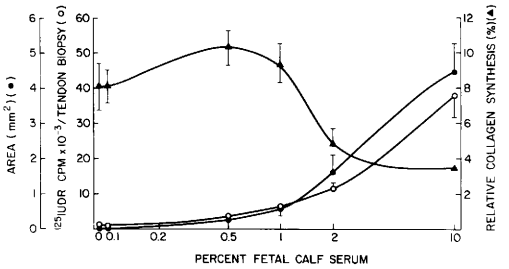

FIG. 2. Effect of FCS on fibroplasia. Various concentrations of whole FCS were used to determine the effect on fibroblast proliferation, migration, and collagen synthesis. Cultures were incubated for 5 days with the indicated concentrations of FCS. Fibroblast proliferation as measured by ^{125}IUDR incorporation into DNA was made on four replicate specimens and the background radioactivity observed in the hydroxyurea blanks (two replicates) was subtracted. Relative collagen synthesis was measured in triplicate and migration was measured using six replicates. The error bars indicate the SEM.

Effects of platelet lysate on fibroblast proliferation, migration, and collagen synthesis. The addition of platelet lysate to medium containing 5% platelet-poor plasma resulted in a proliferative response at low concentrations of lysate (5 μg/ml), approaching a maximal response at 20 μg/ml (Fig. 3). A migratory response, in contrast, did not occur at low concentrations of platelet lysate and was elicited only at the highest concentration of platelet lysate tested (100 μg/ml). This disparity

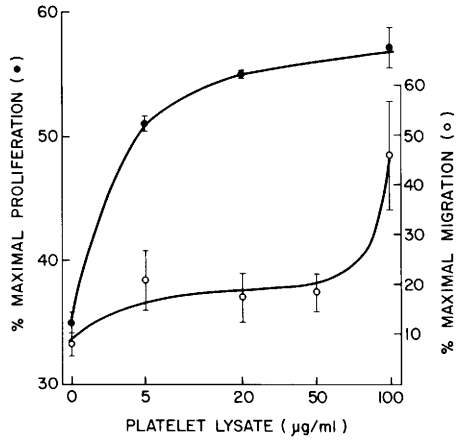

FIG. 3. Effect of PL on fibroblast proliferation and migration. Cultures were incubated for 5 days with the indicated concentrations of PL. fibroblast proliferation was measured by ^{125}IUDR incorporation into DNA (four replicate specimens) and a hydroxyurea blank value (two replicates) was subtracted. Migration was measured using six replicates and error bars indicate the SEM. Data are expressed as percent maximal proliferation observed using 10% FCS (37×10^{-3} cpm/tendon biopsy) and maximal migration observed using 10% FCS (5.1 mm^2).

between proliferative and migratory responses contrasted sharply with the marked similarity between these two events in response to fetal calf serum (Fig. 2). There was no significant effect of varying concentrations of PL on either absolute or relative collagen synthesis in these experiments. Incubation with 10% FCS (positive control) resulted in a relative collagen synthesis value of 6.7% whereas the mean value for PL from 0 to 100 μg/ml was 6.1 ± 0.4%.

Discussion. The most realistic setting for the study of fibroplasia in response to inflammation is obviously an *in vivo* one. However, the multiplicity of factors involved and cellular events occurring precludes that system from being a controllable one for study. Currently, there is considerable debate concerning the validity of cell culture techniques for such studies (26–28). The controversy hinges on the appreciation now that the behavior of fibroplastic cells is governed to a great extent by their extracellular environment and surrounding matrix (8). Any aberration in this environment will undoubtedly affect the cellular events being studied, and hence influence the particular response being documented. In addition, many essential wound elements are not present in the culture dish, again modifying the results. The design of this model for the study of the interrelationship between inflammatory factors and fibroplastic events endeavored to satisfy three criteria: (i) the fibroplastic cells under study should be within their native tissue matrix; (ii) the cells should be exposed to an inflammatory environment that contains the essential elements of a wound; and (iii) all three fibroblast events—proliferation, migration, and collagen synthesis—should be studied under identical conditions, hence permitting a determination of the interrelationship between the events.

The first criterion was satisfied by using tissue explants in culture. The problem of controlling for the different cell types in whole tissue was resolved by employing tendon tissue for the model. Tendon was ideal as it is constituted by a matrix rich in highly organized collagen and a single homogeneous population of fibroplastic cells—tenoblasts. All cellular events observed can thus be attributed to the tendon fibroblasts.

The second criterion was met by surrounding the tissue with a fibrin matrix. Previous studies using this model demonstrated that fibrin was an essential constituent of the plasma clot needed for the rapid migration of tendon fibroblasts out of the tissue (10, 11). The presence of a fibrin matrix resulted in a 54% increase in tendon fibroblast proliferation and a 7- to 10-fold increase in cell migration from the tendon explant (10, 11). Fibroblasts normally migrate on a solid, collagenous, or fibrous matrix of connective tissue or plasma clot and, therefore, their movements should be studied in a solid medium. The peri-explant fibrin matrix employed in this model provides this necessary "scaffold" and we think is an essential component of the cellular environment in studies of this nature.

The third criterion has been satisfied by the model design. Adaptation of established assays for DNA (12, 18–21) and collagen synthesis (22) and an innovative assay for quantifying the area of migration out of the tissue explant allow all three cellular events to be studied in the same preparation (10). This capacity to assay three separate fibroplastic events under identical conditions has provided a valuable

insight into their interrelationships and has also demonstrated their individualistic modes of response. Low concentrations of fetal calf serum (0.1 to 0.5%) favored production of collagen relative to total protein synthesis but were suboptimal for tendon fibroblast proliferation and migration (Fig. 2). In contrast, 10% fetal calf serum was highly stimulatory for proliferation and migration at the expense of differential collagen synthesis (Table I).

The decrease in relative collagen synthesis at high concentration of FCS has been previously reported (27, 28) but was not analyzed by those investigators in relation to maximal fibroblast proliferation or migration. It is understandable that during maximal proliferation and migration, utilization of energy and precursors for synthesis of various cellular components (DNA and cytosol elements) may occur at the expense of other specialized cellular functions (collagen synthesis). It is interesting that in this specialized collagen-producing cell, synthesis of noncollagen protein takes precedence over collagen synthesis in response to 10% fetal calf serum (Table I). In the wound setting, it is likely, however, that despite the decrease in the relative commitment of protein synthesis to collagen, the total amount of collagen being produced is actually increased due to (i) increase in collagen synthesis per cell, (ii) the recruitment of fibroblasts into the area, and (iii) their proliferation.

Platelet-derived growth factor, a known commitment factor for cell proliferation (29–31), was demonstrated to have an effect on fibroblast proliferation at relatively low concentrations, but a migratory effect only at higher concentrations (Fig. 3). The discordance of the proliferative and migratory responses demonstrates either the coexistence of two separate response mechanisms for the two events, or the presence of two distinct proliferative and migratory factors in the platelet lysate. Platelet lysate and platelet-derived growth factor have been shown to be chemotactic for smooth muscle cells (32) and fibroblasts (33) using the Boyden chamber assay. Evidently, platelet lysate, as tested under the conditions in this model, does not contain factors affecting collagen or total protein synthesis.

We present here a model for the study of fibroplasia that now provides a means of defining more clearly the influence of various inflammatory cells and their products on fibroplastic events. Based upon these initial studies, it has become apparent that the mode and the degree of the response of the fibroplastic cells are dependent both on the nature and concentration of the inflammatory factors and possibly a reciprocal relationship between the different cellular events.

This work was supported by NIH Grant GM-20298. The authors thank Fay Akers and Rae Carole Spivey for expert secretarial assistance in preparing the manuscript and to Mrs. Patricia S. Keathley for fine technical assistance.

1. Ross R. The fibroblast and wound repair. Biol Rev **43**:51–95, 1968.
2. Ross R. Wound healing. Sci Amer **220**:40–50, 1969.
3. Schilling J. Wound healing. Surg Clin N Amer **56**:859–874, 1976.
4. Hunt TK, Halliday B. Inflammation in wounds: From "laudable pus" to primary repair and beyond. In: Hunt TK, ed. Wound Healing and Wound Infection. New York, Appleton–Century–Crofts, pp281–293, 1980.
5. Boyden S. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leukocytes. J Exp Med **115**:453–466, 1962.
6. Leibovich SJ, Ross R. A macrophage-dependent factor that stimulates the proliferation of fibroblasts *in vitro*. Amer J Pathol **84**:501–513, 1976.
7. Wahl SM, Whal LM, McCarthy JB. Lymphocyte-mediated activation of fibroblast proliferation and collagen production. J Immunol **121**:942–946, 1978.
8. Hay ED. Cell Biology of Extracellular Matrix. New York, Plenum, 1981.
9. Pickart L. The use of glycylhistidyl-lysine in culture systems. In Vitro **17**:459–466, 1981.
10. Graham MF, Becker H, Cohen IK, Merritt W, Diegelmann RF. Intrinsic tendon fibroplasia: Documentation by *in vitro* studies. J Orthop Res **1**:251–256, 1984.
11. Becker H, Graham MF, Cohen IK, Diegelmann RF. Intrinsic tendon cell proliferation in tissue culture. J Hand Surg **6**:616–619, 1981.
12. Cohen AM, Burdick JE, Ketcham AS. Cell-mediated cytotoxicity: An assay using ^{125}iododeoxyuridine-labeled target cells. J Immunol **107**:895–898, 1971.
13. Hartmann KV, Heidelberger CJ. Studies on fluorinated pyrimidine: XIII. Inhibition of thymidylate synthetase. J Biol Chem **236**:3006–3013, 1961.
14. Munro HN, Fleck A. The determination of nucleic acids. In: Glick D, ed. Methods of Biochemical Analysis. New York, Wiley, pp113–176, 1966.
15. Schmidt G, Tanhauser SJ. A method for the determination of deoxyribonucleic acid, ribonucleic acid

and phosphoproteins in animal tissues. J Biol Chem **161**:83–89, 1945.
16. Mathieson AR, Porter MR. Fractional precipitation of nucleic acid. Nature (London) **173**:1190–1191, 1954.
17. Pfeiffer SE, Tolmach LJ. Inhibition of DNA synthesis in HeLa cells by hydroxyurea. Cancer Res **27**:124–129, 1967.
18. Commerford SL. Biological stability of 5-iodo-2'-deoxyuridine labeled with iodine-125 after its incorporation into the deoxyribonucleic acid of the mouse. Nature (London) **205**:949–950, 1965.
19. Seigers MP, Feinendegen LE, Lahiri S, Cronkite E. Proliferation kinetics of early hemopoietic precursor cells with self-sustaining capacity in the mouse, studied with 125-I-labeled iodo-deoxyuridine. Exp Hematol **7**:469–482, 1979.
20. Diegelmann RF, Cohen IK, Kaplan AM. Effect of macrophages on fibroblast DNA synthesis and proliferation. Proc Soc Exp Biol Med **169**:445–451, 1982.
21. Inoue T, Bullis JE, Cronkite EP, Hüber GE. Relationship between number of spleen colonies and ^{125}IdUrd incorporation into spleens and femur. Proc Natl Acad Sci USA **80**:435–438, 1983.
22. Peterkofsky B, Diegelmann RF. The use of a mixture of proteinase-free collagenases for the specific assay of radioactive collagen in the presence of other proteins. Biochemistry **10**:988–994, 1971.
23. Diegelmann RF, Peterkofsky B. Collagen biosynthesis during connective tissue development in chick embryo. Dev Biol **28**:443–453, 1972.
24. Pledger WJ, Stiles CD, Antoniades HN, Scher CD. Induction of DNA synthesis in BALB/c 3T3 cells by serum components: Re-evaluation of the commitment process. Proc Natl Acad Sci USA **74**:4481–4485, 1977.
25. Warburg O, Christian W. Isolienung und kristallisation des gärungsferments enolase. Biochem Z **310**:384–442, 1941.
26. Peterkofsky B, Prather WB. Increased collagen synthesis in Kirsten sarcoma virus-transformed BALB 3T3 cells grown in the presence of dibutyryl cyclic AMP. Cell **3**:291–299, 1974.
27. Schwarz R, Colarusso L, Doty P. Maintenance of differentiation in primary cultures of avian tendon cells. Exp Cell Res **102**:63–71, 1976.
28. Schwarz RI, Bissell MJ. Dependence of the differential state on the cellular environment: Modulation of collagen synthesis in tendon cells. Proc Natl Acad Sci USA **74**:4453–4457, 1977.
29. Balk SD. Calcium as a regulator of the proliferation of normal but not of transformed chicken fibroblasts in a plasma-containing medium. Proc Natl Acad Sci USA **68**:271–275, 1971.
30. Kohler N, Lipton A. Platelets as a source of fibroblast growth-promoting activity. Exp Cell Res **87**:297–301, 1974.
31. Rutherford RB, Ross R. Platelet factors stimulate fibroblasts and smooth muscle cells quiescent in plasma serum to proliferate. J Cell Biol **69**:196–203, 1976.
32. Grotendorst GR, Seppä HEJ, Kleinman HK, Martin GR. Attachment of smooth muscle cells to collagen and their migration toward a platelet-derived growth factor. Proc Natl Acad Sci USA **78**:3669–3672, 1981.
33. Seppä H, Grotendorst G, Seppä S, Schiffman E, Martin GR. Platelet-derived growth factor is chemotactic for fibroblasts. J Cell Biol **92**:584–588, 1982.
34. Graham MF, Diegelmann RF, Cohen IK. Effects of inflammation on wound healing. I. In vitro studies. In: Hunt TK, Heppenstall RB, Pines E, Rovee D, Eds. Soft and Hard Tissue Repair: Biological and Clinical Aspects. New York, Prager, Chap 20, 1984.

Received September 8, 1983. P.S.E.B.M. 1984, Vol. 176.
Accepted March 27, 1984.