spring, a statistically significant difference (χ^2, $P < .01$).

In response to the discovery of the significant number of "light for dates" births in the prednisone-exposed group of human infants, a second analysis of variance was performed to determine if the significant difference between the experimental and control groups would persist with the extremely low-weight subjects removed. The analysis, with "light for dates" subjects eliminated from both prednisone and control groups, showed that the hormone-exposed subjects were still significantly lighter than controls ($P < .0001$) (see Fig. 1). This finding indicates that, although only 16 prednisone-exposed subjects were born at weights which fit the strictly defined criterion for "light for dates," the majority of the experimental subjects were stillborn at weights lighter than would be expected. To illustrate the magnitude of intrauterine growth retardation in the prednisone-exposed offspring, it was determined that with "light for dates" subjects removed from both conditions, 60 percent of the remaining hormone-exposed individuals fell below the 25th percentile of the unexposed group.

The retardation of intrauterine growth after exposure to prednisone indicates that exogenous adrenocortical hormones can have a marked effect on fetal development. We therefore suggest that physicians be alerted to the possible at-risk status of babies born to prednisone-treated mothers, regardless of apparently normal birth weights and lengths of gestation.

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9. Prednisone, a synthetic corticosteroid with a potency five times that of cortisone, has become the steroid of choice in clinical practice [see McMahon (*1*); H. Jick, *Drug Ther.* **5**, 85 (1975)].
10. Rockland-Swiss albino mice, orginally derived from stock maintained by M. X. Zarrow, were kept as an outbred strain in a closed colony.
11. Day 13 was selected for the initiation of treatment in order to guarantee the health of the dam, the viability of the pregnancy, and the integrity of the offspring. We have found that the risk of implantation failure, fetal resorptions, spontaneous abortions, stillbirths, and congenital anomalies is markedly increased when treatement is begun prior to this time.
12. Steroid suspending vehicle was donated by the National Cancer Institute.
13. Fourteen-day-old fetuses cannot be sexed by external morphological indices.
14. For data on humans, see I. Z. Beitirs, F. Bayard, I. G. Ances, A. Kowarski, C. J. Migeon, *J. Pediatr.* **81**, 936 (1972); C. J. Migeon, J. Bertrand, C. A. Gemzell, *Rec. Prog. Horm. Res.* **17**, 207 (1961); C. J. Migeon, H. Prystowsky, M. M. Grumbach, M. C. Bryon, *J. Clin. Invest.* **35**, 488 (1956). For data on animals, see S. Milkovic, K. Milkovic, I. Sencar, J. Paunovic, *Prog. Brain Res.* **32**, 71 (1970); W. Waddell, *Teratology* **5**, 219 (1972); M. X. Zarrow, J. E. Philpot, V. Denenberg, *Nature (London)* **226**, 1058 (1970).
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19 April 1978; revised 23 June 1978

Hepatic Fibrosis in Schistosomiasis: Egg Granulomas Secrete Fibroblast Stimulating Factor in vitro

Abstract. *Cytosol extracts and culture supernatants of isolated egg granulomas obtained from livers of mice with* Schistosoma mansoni *infection stimulated fibroblasts to incorporate tritiated thymidine and to proliferate in vitro. This finding suggests that hepatic granulomas may play a role in regulating hepatic fibrosis in* Schistosoma mansoni *infections.*

Hepatic fibrosis may be initiated by a variety of insults, including toxins and infection, and can result in portal hypertension and gastrointestinal hemorrhage. In infections due to the trematode worms *Schistosoma mansoni* and *S. japonicum*, hepatic fibrosis is the major cause of morbidity and mortality. The development of hepatic fibrosis in schistosomiasis is preceded by formation of granulomas surrounding the trematode eggs which become trapped in the portal venules of the liver. The development of these granulomas is similar in humans and mice infected with *S. mansoni*. In mice the granulomatous reaction has been shown to represent a delayed hypersensitivity response to soluble egg antigens (*1*). Here we report results of our investigations that suggest that products of schistosomal granulomas can stimulate fibroblasts in vitro and therefore may play a role in the development of hepatic fibrosis in schistosomiasis.

Lymphocytes elaborate soluble substances in vitro that are capable of inhibiting (*2*) or enhancing (*3*) fibroblast proliferation, and of stimulating collagen synthesis in vitro (*2*, *3*). Macrophages also influence fibroblast growth and protein synthesis (*4*, *5*). Since lymphocytes and macrophages constitute the predominant cell population in the prefibrotic granuloma, we investigated whether these granulomas might also regulate fibroblast activation in vitro. To do so, we prepared isolated schistosomal granulomas by a modification of the technique described by Pellegrino and Brener (*6*). This technique separates granulomas from liver homogenates by repeated sedimentation at 1*g*; free hepatic parenchymal cells, cell stroma, and cell debris remain in the supernatant and are discarded.

Outbred white mice (National Institutes of Health general purpose) were infected subcutaneously with approximately 500 cercariae of *S. mansoni* [Puerto Rico I strain (*7*)] obtained by passage through the snail vector (*Biomphilaria glabrata*). Seven weeks later, livers from individual mice were homogenized in a Waring blender for 1 to 2 minutes in Hanks buffer (Hanks balanced salt solution) and granulomas

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were isolated by repeating the 1*g* sedimentation three times in Hanks buffer. The granulomas prepared in this manner contained mononuclear cells circumferentially arranged around a schistosomal egg. Occasional eosinophils and fibroblasts were also identified in some granulomas. Small numbers of hepatic parenchymal cells were present on the rim of some of the granulomas.

In the initial studies we prepared cytosol extracts from isolated granulomas and from homogenates of normal livers from uninfected mice. Unfractionated homogenates of normal liver and isolated granulomas suspended in RPMI 1640 medium were subjected to a one-step freeze-thaw (−80° to 20°C) procedure, followed by brief sonication (2 A, d-c output for 90 seconds). Supernatants were obtained after sonication by centrifugation (400*g* for 10 minutes) and dialyzed against medium for 24 hours at 4°C. They were then sterilized by filtration and tested for their effects on the incorporation of [^{3}H]thymidine in vitro by monolayer cultures of guinea pig dermal fibroblasts (*3*). Cultured fibroblasts were diploid and retained the ability to synthesize collagen in vitro. These experiments revealed that granuloma extracts tested over a wide range of concentrations stimulated fibroblasts to incorporate [^{3}H]thymidine (Fig. 1). Significant fibroblast stimulating activity was noted in extracts diluted greater than 1:2 in medium. Maximum stimulation, which resulted in a 16-fold increase in [^{3}H]thymidine incorporation above control (medium alone), was obtained with extracts diluted 1:10. In contrast, extracts of normal liver tested at the same dilutions contained little or no fibroblast stimulating activity. These observations suggested that the granulomas but not liver parenchymal cells contained a substance that could stimulate fibroblasts to incorporate [^{3}H]thymidine in vitro.

To determine whether intact isolated granulomas could elaborate the fibroblast stimulating factor in vitro, we incubated granulomas from individual mice in serum-free RPMI 1640 medium for 48 hours, after which we obtained the supernatants by centrifugation (200*g* for 10 minutes). Culture supernatants were dialyzed for 24 hours against medium and tested in fibroblast cultures. Dialyzed and in some cases diluted nondialyzed supernatants stimulated fibroblasts (Fig. 2). Dermal fibroblast cultures derived from C57Bl mice could be stimulated in a similar manner. In addition, dialyzed supernatants stimulated increased incorporation of [^{3}H]thymidine into trichloroacetic acid precipitable material in fibroblast cultures. Since granuloma supernatants also stimulated proliferation of fibroblasts as assessed by cell counts ($25 \pm 0.5 \times 10^4$ cells per stimulated culture as opposed to $6 \pm 0.3 \times 10^4$ cells per control culture), it appears that the increases in [^{3}H]thymidine incorporation by fibroblast cultures is a measure of fibroblast proliferation. Our observations therefore suggest that isolated granulomas can elaborate in vitro a factor that stimulates fibroblast proliferation. It is likely that fibroblast stimulating factor was elaborated into culture medium by living granuloma cells in these experiments, because granulomas continued to produce this factor for at least 96 hours and because culture viability was confirmed by ongoing incorporation of labeled amino acids.

These findings suggest that in addition to their ability to elaborate specific medi-

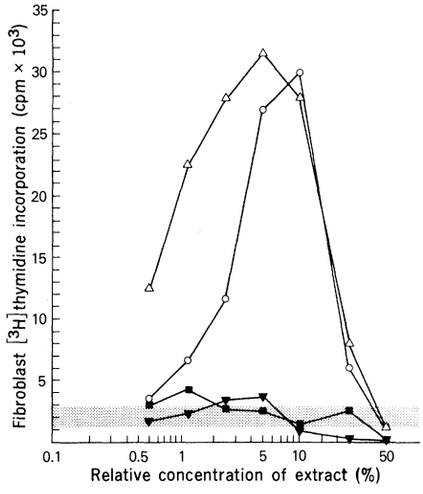

Fig. 1 (left). Effects of cytosol extracts of normal liver tissue (solid squares and triangles) and of isolated schistosomal granulomas (open triangles and circles) on the incorporation of [^{3}H]thymidine by fibroblasts in vitro. Isolated granulomas from individual mice were washed thrice in Hanks buffer and placed in RPMI 1640 to make a 10 percent suspension. Livers from individual age-matched uninfected mice were homogenized (Waring blender, 1 minute) in RPMI 1640 (one liver per 100 ml). Extracts were obtained from these preparations by a freeze-thaw procedure and sonication, and the filter-sterilized supernatants obtained by centrifugation (400*g*, 20 minutes) were tested at different concentrations in fibroblast cultures. Monolayers of dermal fibroblasts (Hartley guinea pig) were derived from confluent cultures by trypsinization and incubated at a concentration of 10^5 cells per milliliter in serum-free Dulbecco-Vogt medium in a volume of 1 ml in each 1-dram glass vial with or without the addition of extracts. Cultures were maintained for 72 hours at 37°C, in an atmosphere of 95 percent air and 5 percent CO_2; 1 μCi of [^{3}H]thymidine (specific activity 6.0 Ci/mmole; Schwartz/Mann) was added to each vial for the final 4 hours of incubation. Fibroblasts were harvested on glass fiber filters and the magnitude of [^{3}H]thymidine incorporation was determined by scintillation counting. The mean of triplicate determinations in separate representative experiments is shown; the standard error of the mean was ≤ 10 percent in all cases. The horizontal stippled bar encompasses the mean counts per minute (± standard error) of fibroblasts cultured in medium alone.

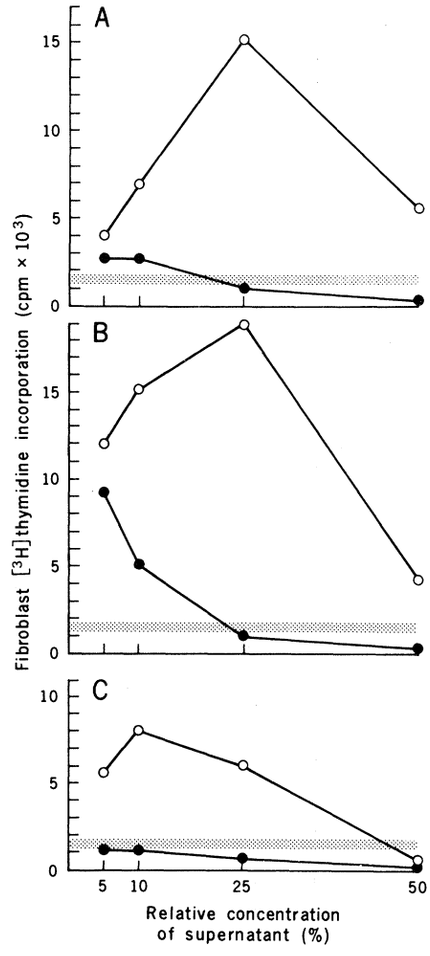

Fig. 2 (right). Effects of dialyzed (open circles) and nondialyzed (solid circles) granuloma culture supernatants at different concentrations on the incorporation of [^{3}H]thymidine by fibroblasts in vitro. Isolated granulomas from individual animals were suspended at a concentration of approximately 20 to 50 per milliliter in serum-free RPMI 1640 containing antibiotics. Twenty milliliters of suspension were incubated in each 250-ml plastic tissue culture flask (No. 3075, Costar) at 37°C in an atmosphere of 95 percent air and 5 percent CO_2 for 48 hours. Portions of culture supernatants were then dialyzed against medium for 24 hours at 4°C. Filter-sterilized supernatants were tested in vitro for their effects on [^{3}H]thymidine incorporation by fibroblasts. The mean of triplicate determinations in three representative experiments (A to C) are shown; the standard error was ≤ 10 percent in all cases. The stippled horizontal bar encompasses the mean (± standard error) of [^{3}H]thymidine incorporation by fibroblasts cultured in medium alone.

ators of inflammation, such as migration inhibition factor (*8*) and eosinophil stimulation promoter (*9*), isolated schistosomal granulomas from mice can elaborate substances that influence fibroblast activation. We have provided evidence here for the presence of molecules within granulomas and their culture supernatants which can stimulate fibroblast proliferation in vitro. In related studies, we have found that these granuloma supernatants induce changes in the intracellular concentrations in fibroblasts of adenosine 3′,5′-monophosphate and prostaglandins, and alter the ability of fibroblasts to incorporate [^{3}H]proline into collagen. Although it seems likely that the source of the stimulating factor is the inflammatory cells, since mononuclear cells have been shown capable of producing related substances (*2–5*), we cannot at present exclude the possibility that it might derive from the schistosomal egg.

These data suggest a possible molecular link between granuloma formation and the subsequent development of hepatic fibrosis in *S. mansoni* infections. Since the egg granuloma in this disease appears to represent a chronic inflammatory response to egg antigen (*1*), the biological implications of these findings potentially extend to fibrosis resulting from chronic inflammation in a variety of other conditions as well.

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10. We thank A. W. Cheever of the National Institutes of Health for providing infected mice, for reviewing the manuscript, and for encouragement and interest. L. C. Koontz and J. B. McCarthy provided excellent technical assistance.

30 December 1977; revised 17 May 1978

Selective Inhibition of Tubulin Synthesis by Amiprophos Methyl During Flagellar Regeneration in *Chlamydomonas reinhardi*

Abstract. *Amiprophos methyl (APM) is a strong, readily reversible and highly selective inhibitor of tubulin synthesis in* Chlamydomonas reinhardi. *The extensive induction of tubulin synthesis that accompanies flagellar regeneration in this organism is prevented by 3 to 10 μM APM. When applied after induction has begun, APM causes a rapid cessation of tubulin synthesis. Translation studies in vitro indicate that the lack of tubulin production in APM-treated cells is not due to a direct inhibition of tubulin messenger RNA translation but rather to a selective depletion of tubulin messenger RNA.*

Microtubules play a central role in several cellular processes (*1*, *2*) including cell division, motility, cell surface organization, secretory functions, cellular differentiation and, perhaps, control of cell proliferation (*3*). A great deal has been learned regarding the molecular properties of microtubules and their assembly (*1*), yet relatively little is known concerning the mechanisms responsible for controlling the synthesis of the tubulin subunits from which microtubules are assembled. One process in which tubulin synthesis has been shown to be intimately involved is the regeneration of flagella in *Chlamydomonas reinhardi* (*4*, *5*). In this system the removal of flagella leads to a rapid and extensive induction of tubulin synthesis. An early observation relating to the control of tubulin synthesis in these cells was that treatment with colchicine or other compounds which prevented flagellar regeneration, and thus blocked utilization of cytoplasmic tubulin reserves (a potential feedback signal), did not interfere with the induction of tubulin synthesis (*4*, *5*). We have now tested for its effects on tubulin synthesis, another compound which has recently been shown to inhibit flagellar outgrowth (*6*). In contrast to the previous inhibitors, this compound, APM (amiprophos methyl) (Fig. 1) (*7*), had a marked inhibitory influence on tubulin production in deflagellated cells. Indeed, subsequent experiments have shown that APM is a rapid, reversible, and selective inhibitor of tubulin synthesis in *C. reinhardi*.

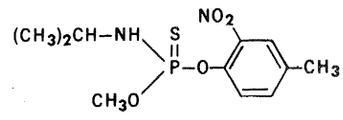

Fig. 1. Amiprophos methyl [APM; *O*-methyl-*O*-(4-methyl-6-nitrophenyl-*N*-isopropyl-phosphorothioamidate].

Gametic cells of strain 137c were obtained by overnight incubation of vegetative cells in a nitrogen- and sulfate-deficient medium (medium V) as described (*5*). For analysis of tubulin induction, cells were deflagellated by incubation at *p*H 4.7 for 1 minute (*8*) and labeled for 30-minute intervals with $H_2{}^{35}SO_4$. Labeling procedures were modifications (*5*) of those developed by Lefebvre and Rosenbaum (*9*); we used 100 μCi of carrier-free $H_2{}^{35}SO_4$ to label approximately 10^7 cells in 0.5 ml of medium V. After labeling, cells were precipitated with ten volumes of acetone and dried. The acetone powders were dissolved in 2 percent sodium dodecyl sulfate, 5 percent mercaptoethanol, 10 percent glycerol, 60 m*M* tris (*p*H 7.6), and a portion (containing 2×10^6 count/min) was subjected to electrophoresis (except where noted) on a 9 percent polyacrylamide gel containing sodium dodecyl sulfate. Electrophoretic conditions were as described (*4*, *5*).

When gametic cells of *C. reinhardi* were deflagellated, tubulin synthesis began within 15 minutes and continued at high levels (15 to 20 percent of total cellular protein synthesis) for approximately 1 1/2 hours (*4*, *5*) (Fig. 2A, tracks 1 to 4). If these cells were treated with 3 μ*M* APM 5 minutes prior to deflagellation, tubulin induction was inhibited (Fig. 2A, tracks 5 to 8). In this experiment only small quantities of tubulin synthesis were detected in the first (15 to 45 minutes) labeling period after deflagellation and only trace amounts in the next or subsequent periods. Thus, APM has a rapid and severe effect on tubulin induction after deflagellation.

In addition to blocking the initial induction of tubulin synthesis, APM also caused a rapid cessation of tubulin synthesis when applied to cells at any time during the inductive cycle. For instance, if induction was allowed to proceed for 15 minutes prior to administration of APM, tubulin synthesis in the succeeding 30-minute period (Fig. 2B, track 1) was greatly reduced compared to that in nontreated cells (Fig. 2A, track 1). In the following labeling period little or no tubulin synthesis is evident (Fig. 2B, track 2). Even more dramatic were the

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