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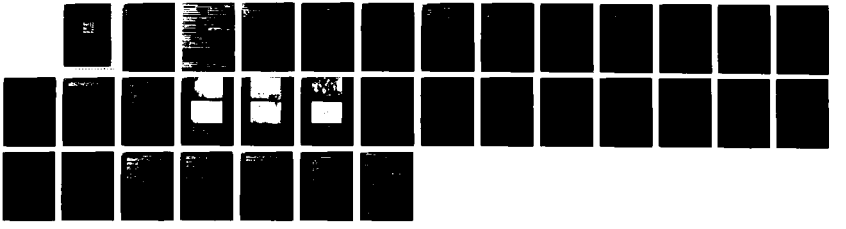

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Role of Fibronectin in Wound Healing

Annual/Final Report

Andy C. Reese

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The purpose of the project was to determine if local or systemic manipulation of circulating fibronectin (Fn, a normal plasma and extracellular matrix glycoprotein) levels affects the rate of wound healing. Initial experiments were designed to determine if Fn is involved in opsonization of effete cells and tissue debris for removal by tissue macrophages. Within two hours after wounding and injection of fluorescene-labeled Fn, tissue debris at the wound site was coated with Fn, and dammaged cells were also coated by 24 hrs. By 48 hrs, macrophages at the site of injury had phagocytized Fn coated tissue debris and/or cells. Thus, effete materials at the wound site are quickly labeled by plasma Fn for removal by tissue macrophages.

Subsequent experiments were done to determine if Fn enhanced the healing rate of dermal injuries. Rat Fn was suspended in various inert carriers and used to treat full thickness skin lesions on rats. Fn in several carriers was effective in stimulating significantly faster wound healing than was seen with the carrier mixed with PBS. Further

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[illegible] showed that treatment once a day for two days was as effective in enhancing [illegible] as more prolonged treatment. A single treatment with Fn on the day of the [illegible] enhanced wound healing but not as much as treatment for two days. One could also [illegible] starting treatment for a few hours after injury and still significantly improve the healing rate.

When rats were given cyclooxygenase inhibitors prior to surgery, surgery induced Fn depression was abrogated. Rats maintained on an essential fatty acid free diet were also resistant to surgery induced reductions in circulating Fn. Injection of either thromboxane or prostacyclin reduced Fn levels. Further development of these results may lead to ways to manipulate circulating Fn levels for therapeutic benefits.

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory animals of the Institute of Laboratory Animal resources, National Research Council (HEW Publication No. (NIH) 78-23, revised 1978).

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BODY OF REPORT

Statement of the Problem:

Many of the physiological activities of fibronectin (Fn); e.g., stabilization of blood clots, providing anchorage points for macrophages and fibroblasts, chemotactic activity, etc., are consistent with its having an important role in reestablishing homeostasis following trauma (Reviewed in Reese et al., 1983). However, circulating Fn levels are depressed following traumatic injury, so immediately following an injury, Fn may not be available from circulation in sufficient quantity to effectively carry out all of these functions. Therefore, it was important to directly examine the possibility that treatment with exogenous Fn could enhance healing of external injuries. If these injuries respond to exogenous Fn, it would be logical to assume that healing of internal injuries would be benefited by increasing the Fn available to the injury; i.e., the circulating level of Fn. The plasma levels of Fn available to internal injuries may be increased by three means: 1) infusing additional Fn, 2) preventing the depression of circulating Fn following the initial trauma, 3) stimulating synthesis of additional Fn following trauma. The first of these is being tried by several laboratories (reviewed by Doran et al., 1986). Knowledge of the mechanism by which plasma Fn levels are depleted following injury may lead to methods of preventing or reducing the depression.

Background:

Fn is a 440,000 dalton glycoprotein which is a normal constitutent of plasma and the extracellular matrix. There is a species and sex variation in plasma concentrations; males tend to have levels about 10-15% above those of females, and rat concentrations (about 400 ug/ml) are a little higher than those in humans (about 300 ug/ml). Many of Fn's activities can be traced to its adhesive qualities. It serves as an anchorage point for the binding of various cells to the extracellular matrix, blood clots, tissue debris, and each other (Mosher 1984). Because of this property, it is important in reticuloendothelial system (RES) function (its removal results in RES blockade), synersis of blood clots and wound contracture, and integrity of the epidermis (epithelial cells bind to the Fn in the extracellular matrix) (summerized in Yamada, 1983). In addition, Fn and its fragments are chemotactic for fibroblasts, (Knox et al., 1986), monocytes (Norris et al., 1982), and epithelial cells (Donaldson and Mahan, 1983), inducing them to move into the injured area and controlling the movement of neural crest cells during embryogenesis (Bonner-Fraser, 1985).

It seems probable that Fn is involved in each of the various stages of wound healing. 1) It makes up about 4-5% of the blood clot (Mosher, 1980) and is cross-linked to the fibrin fibers by coagulation Factor XIIIA (Mosher et al., 1980). The Fn incorporated in the clot serves as attachment sites for macrophages and later fibroblasts to move into the clot along the fibrin scaffolding. 2) Before any wound can heal, macrophages must remove the dead cells, cellular debris, blood clot, and some extracellular material (Bennaceraf et al., 1975; Saba, 1970). Since Fn serves as an opsonin for removal of circulating cell debris by the fixed cells of the RES (Saba, 1970) and Fn binds to aggregated cell membranes (Molnar et al., 1977), actin (Keski-Oja and Yamada, 1981), and collagen

(Engvall and Ruoslahti, 1977), it seems likely that tissue macrophages (which have receptors for Fn [Bovilaqua et al., 1979]) also recognize Fn as an opsonin on the tissue debris at the site of the injury. 3) Repair of the injury begins when fibroblasts start to move into the area and secrete collagen and other extracellular materials. Fragments of Fn are known to be chemotactic for fibroblasts (Knox et al., 1986) and probably are at least partially responsible for their movement into the wound. In addition, fibroblasts synthesize and secrete Fn along with collagen. The Fn cross-links the collagen fibers and contributes to the stability of the matrix.

Plasma Fn levels are very trauma sensitive, the amount of the decrease being proportional to the extent of injury (Lanser et al., 1980) following blunt and operative trauma, burns, intravascular coagulation, advanced cancer, etc. (reviewed in Reese et al., 1983). The depression has been attributed to consumption of Fn by binding to tissue debris at the wound site (Saba and Scovill, 1975), but the amount of Fn that would have to bound at the site of even a simple surgical incision to produce the observed decline makes it likely that additional processes are involved. In addition, the speed with which plasma Fn rebounds to supranormal levels suggests that there is some replacement form sequestered Fn (Reese et al., 1982). Since prostaglandin levels (particularly thromboxane A_2) are increased by each of the procedures or conditions which depress Fn concentrations and given the importance of prostaglandins as regulators of many physiological processes (Cook et al., 1980), it is possible that one or more of the prostaglandins may be involved in the trauma induced depression of circulating Fn levels.

Given the evidence of Fn's involvement with wound healing and its depression following trauma, it was a logical extension to use Fn replacement therapy to treat patients with traumatic injury. There have been anecdotal reports of dramatic improvement in septic trauma patients following infusion of Fn (Saba et al., 1978; Robbins et al., 1981). However, more controlled studies have not been as hopeful (Lundsgaard-Hansen et al., 1985). Nevertheless, a means of regulating Fn levels by manipulating the internal controls rather than by exogenous administration of Fn may provide a useful new tool in the physician's armamentarium.

Methods:

Animals--Sprague-Dawley rats were used as both experimental animals (250-350g) and the source of Fn (retired breeders). They were maintained in our vivarium and, except where otherwise indicated, were given Purina Lab Chow and water ad libitum. All experiments were conducted in accordance with guidelines established by NIH and were reviewed by the Medical College of Georgia's Comittee on Animal Use in Research and Education.

Fibronectin Purification--Fn was purified by a modification (Doran et al., 1980) of the method of Engvall and Ruoslahti (1977). Briefly, citrated plasma was incubated in batch with gelatin-Sepharose for 2 hrs at room temperature. The slurry was then poured into a column and washed extensively with phosphate buffered saline (PBS) followed by 1 M urea-0.05 M Tris. Fn was eluted from the column with 4 M urea in Tris buffer and vacuum dialyzed against PBS. Purity was checked by polyacrylamide gel electrophoresis and high performance liquid chromatography. Each batch of Fn

was tested for its ability to agglutinate gelatin coated latex beads (Check et al., 1979) as one measure of fun tional activity.

Fluorescent Labeling--fn was coupled to fluorescein isothiocyanate (FITC) and human serum albumin (HSA) was coupled to rhodamine isothiocyanate (RITC) via Goding's procedure (1976).

Wound Treatment--Fn was added to PBS, dimethyl sulfoxide (DMSO), Aquaphor (Beiersdorf Inc., South Norwalk, Conn.), Orabase (Hoyt Labs, Needham, MA), hydrophylic petrolatum, Sepharose 4B (Pharmacia), or polyethylene glycol (Fisher) at a final concentration of 500 or 1000 ug/ml. In initial studies, each rat was treated 3 times daily for twelve days; in subsequent experiments, they were treated using the schedules indicated. The liquid samples were applied a drops from a sterile pasteur pipette; the salves were applied with a cotton swab.

Rebuck Skin Window--One mg of Fn-FITC and HSA-RITC each was injected i.v. into SD rats. (This was equal to approximately 2% of the total circulating Fn.) The rat's abdomen was clipped and shaved. An area about 2 cm^2 was scraped with a scalpel to just remove the dermis taking care not to rupture any surface capillaries. One drop of bacterial cultural supernatant (MCG Pharmacy) containing chemotactic factors was placed on the scraped are which was then covered with a 1 cm^2 glass coverslip. The glass was covered with a small piece of cardboard and held in place with adhesive tape wrapped completely around the animal. After 24 hrs, the coverslips were removed and examined under incident and u.v. light. When macrophages were collected for study, a leucite chamber which had two ports on the side was used in place of the coverslip. (Seal the edges with silicone high vacuum grease.) The chamber was filled with 2 mls of medium containing chemotactic factors. The medium was changed at 24 hrs and collected at 48 hrs. Drops of the medium were placed on slides and examined for fluorescent neutrophils and macrophages.

Standard Lesions--Rats were anesthetized with ether. Their backs were prepared by clipping, shaving, washing with Physohex, and swabbing with an alcohol pad. Four to eight Excised wounds were made by folding the skin on the rat's back and punching a hole through the double thickness with a standard, hand held paper punch. One or two holes (as needed) were used as controls while the remaining holes were used for the experimental treatment as described. The dimensions were measured on the appropriate days, and the calculated areas entered into the following equation:

$$\frac{\text{Area } (mm^2) \text{ of wound on day X}}{\text{Area } (mm^2) \text{ of wound on day 0}} \times 100 = \% \text{ of original wound on day X}$$

The data were analyzed using a two tailed, paired Student's t test.

Surgical Shock--A 2-3 cm abdominal midline incision was made and about 5 cm of the intestine was exteriorized. The intestine was gently kneaded for about 30 sec, then it was replaced in the abdominal cavity. The muscle layer was closed with sutures of 4.0 silk, and the skin with wound clips. The entire procedure was done under deep ether anesthesia.

Results and Discussion:

(Objective--Confirm that Fn opsonizes tissue debris for removal by tissue macrophages)

Fn-FITC (which fluoresces green under u.v. light) was injected i.v. in quantities such that the Fn-FITC would be approximately 2% of the total plasma Fn. Equal amounts of HSA-RITC (which gives red fluorescence) were injected at the same time as a control. Immediately following injection, two Rebuck Skin Windows were made and a 1 cm^2 glass coverslip applied to each. Both coverslips were removed at 24 hrs and were viewed in their entirety under normal transmitted light (Fig. 1A) and u.v. epi-illumination (Fig. 1B) with a microscope. (No quantitation of the labeled vs unlabeled debris was attempted since the primary goal was to determine if any labeling of debris with FITC or RITC had occurred.) Debris showing green fluorescence (bound Fn) was clearly visible on the coverslips. This clearly establishes that circulating Fn is available to bind to the debris at the site of an injury, and therefore, Fn synthesis by fibroblasts and endothelial cells at the wound site is not required for opsonization. No debris showing red fluorescence (bound albumin) was ever found (not shown), indicating that the binding of Fn was specific and not just the result of plasma accumulation at the site due to injury induced inflammation.

In order to determine if Fn is also able to bind to effete cells, coverslips prepared as in Figure 1 were examined until fragments of tissue containing whole cells were found. These were examined under visible (Fig. 2A) and u.v. light (Fig. 2B) as above. The cells are clearly labeled with Fn-FITC, but again no red fluorescence (HSA) was ever seen (not shown). The Fn is binding to the membranes of the effete cells since they all show ring fluorescence.

Macrophages were obtained from the scraped areas 24 to 48 hrs after the injury as described. The arrows in Figure 3A point to the macrophages which are fluorescent under u.v. epi-illulmination shown in 3B. The solid pattern of fluorescence indicate that the macrophages had internalized the Fn-FITC labeled debris. Since none of the macrophages exhibited red fluorescence (HSA-RITC) or yellow fluorescence (both RITC and FITC) (not shown), the green fluorescence cannot have been due to simple pinocytosis of the tissue fluid since they contain both Fn-FITC and HSA-RITC. Since other labs have shown that aggregated cell membranes are not phagocytized unless they are opsonized with Fn (Blumenstock et al., 1981) and that tissue debris must be opsonized with Fn to be cleared from circulation (Molnar et al., 1977), it seems very likely that Fn is a necessary opsonin for wound debridement by macrophages. Polymorphonuclear leukocytes (PMNS) do not seem to contribute to the debridement. Even though they can recognize and phagocytize targets coated with Fn (Raynor et al., 1981), PMNs with internalized Fn-opsonized tissue debris were not seen. This is consistent with the observation of Simpson and Ross (1972) that PMNs are not necessary for wound debridement since neutrophil depletion has no effect on healing time if the wound is kept sterile.

(Objective--Determine if local treatment of wounds with topically applied Fn is efficatious)

Standard excised lesions were prepared on the rats' backs, and the wounds were treated three times each day for 12 days with Fn in the carriers listed in Table I. Fn applied in PBS had no effect, probably because the drop tended to bead up and roll off, therefore the Fn did not remain in contact with the wound long enough to opsonize the debris. In contrast, Fn mixed with DMSO stimulated faster healing than that induced by DMSO alone or with PBS (with or without added Fn). In this and other experiments, DMSO alone had little or no effect on wound healing, which is consistent with the results of Goldblum (1983). (DMSO in the concentration used here has been shown to have no toxic effects [Rubin, 1983]).

Fn suspended in an inert ointment as a carrier would be much easier to apply to wounds than a liquid. However, the first carrier tried, Aquaphor, appeared to inhibit wound healing compared to the control treated with PBS. Aquaphor's inhibitory effect was not reversed by addition of Fn. Although not readily apparent from the data shown here, Orabase by itself seems to stimulate somewhat faster healing. When a concentration of 1000 ug Fn/ml in Orabase was used, there was additional enhancement of healing which was significant at the 0.05 level. Lower Fn concentrations had little effect.

Since Fn in either DMSO or Orabase enhanced wound healing, we wanted to determine if together they would synergise to produce even faster healing. However, when DMSO was mixed with Orabase, there was a reaction which caused the Orabase to clump. The mixture was very difficult to handle and did not cover or stick to the wound well. Therefore, the DMSO-Fn solution mixed with Orabase was ineffective in enhancing the healing rate.

Other carriers were tested using the same treatment schedule. Results of a typical experient are shown in Table II. Fn in each of the carriers significantly enhanced the healing rate. Polyethylene glycol 20000 was most effective. In subsequent tests (not shown), polyethylene glycol and Orabase were equivalent as carriers for Fn in enhancing wound healing.

It was also necessary to determine the most effective treatment schedule. Eight (four pairs) of the standard lesions were made. One wound on each animal was treated with Orabase alone. The remaining wounds were treated once each day with Orabase containing 500 ug Fn/ml. One wound on each animal was treated for 2 days, one for 4 days, one for 6 days, one for 8 days, and one for 10 days. Wounds were measured on days 2,4,6,9, and 11. Except for day 6, all of the treated wound healed significantly faster ($P \leq 0.05$) than the untreated wounds (Table III). However, there was essentially no difference in the subsequent healing between wounds treated for 2 days and those treated for longer periods. (The statistically faster healing rate by day 11 observed with the 2 day treated wounds compared with those treated 4 days or 10 days is probably not physiologically important.) These results were extended to determine if a single treatment with Fn would be effective in speeding wound healing. As can be seen from the bottom protion of Table III, a single treatment with Fn does enhance wound healing essentially equivalent to that of 2 day treatments. Thus, the effect of topical Fn is exerted early, and little or nothing is gained by continued application of Fn beyond the first couple of days.

There have been reports that animals deficient in essential fatty acids are more resistant to shock induction than normals. To determine if this condition has an effect on wound healing and/or on the ability of the body to use externally applied Fn, rats were maintained on an essential fatty acid free diet for 30 (rats weighing less than 200 gm) or 60 (retired breeders weighing greater than 450 gm) days prior to initiation of the wound healing experiments. Two pairs of standard lesions were made on the backs of the rats. One lesion of each pair (the control) was treated with Orabase or DMSO alone and the other (the experimental) with the same carrier containing Fn. Three treatments daily were continued for 12 days to provide the greatest possible time for any effect to be seen. The results shown in Table IV indicate that Orabase-Fn was effective in stimulating wound healing ($P \leq 0.05$), and the effect was apparent within 4 days after beginning treatment. This is quite similar tn both young adult (approximately 4 mo.) and middle aged (approximately 12 mo.) rats. Interestingly, Fn in DMSO does not stimulate any more rapid healing in these animals than DMSO alone. The healing rate of the EFA deficient rats appears to be slightly slower than that of normal rats.

The ability of exogenous Fn to accelerate wound healing is perhaps a little surprising since the debris is labeled by plasma Fn within a few hours of injury and exogenous Fn acts early in the process. During the time period when exogenous Fn is most effective; i.e., for a few hours to 2 days after the injury, the most important healing activities are the migration of the monocytes and fibroblasts into the wound and the debridement of the wound by the macrophages. Exogenous Fn may be the source of extra chemotactic fragments which would result in the more rapid accumulation of monocytes and fibroblasts at the site of the injury. In addition, the affinity of the Fn receptor on macrophages is relatively low (Rollins et al., 1982) so additional coating of the debris may increase the avidity of the binding of the debris to the macrophages.

(Objective--To determine how circulating levels of Fn are controlled)

Although we had previously shown that Fn is actively transported to the injury site in at least some kinds of wounds (Reese et al., 1982), it seemed unlikely that all of the decrease could be due to this mechanism. Since prostaglandin levels are elevated by the same kinds of traumas which lower Fn concentrations and prostaglandins are known to be important in controlling other physiological processes, it seemed reasonable to examine a possible role for prostaglandins in controlling Fn levels.

Essential fatty acid deficient rats lack arachidonic acid which renders them unable to synthesize prostaglandins. Rats were maintained for 4 weeks on the essential fatty acid free diet then used in the wound healing experiments which lasted two weeks. They were maintained for two additional weeks after complete wound healing before being used in the shock experiments. The results of a typical experiment (Table V) indicate that the inability to make prostaglandins also abrogates the surgery induced dip in Fn. Identical results were obtained with young rats ($\frac{1}{2}$ 4 mo. old) and with middle aged rats ($\frac{1}{2}$ 1 yr old). It is possible that this stabilized fibronectin level contributes to the reported resistance of EFAD rats to the lethal effects of shock (Cook et al., 1981).

Surgical trauma results in a decrease in circulating Fn levels. Saba has used a midline incision followed by mild intestinal manipulation as a reproducible means of inducing a depression in plasma Fn concentrations (Saba and Scovill, 1975). In our hands, this technique induces a reproducible 15-20% decrease in plasma Fn within the first 2 hrs followed by a rebound to normal or slightly above normal levels by 24 hrs (Table VI and first 2 lines of Table VII). Indomethacin is a widely used inhibitor of cyclooxygenase and, thereby of prostaglandin synthesis. Table VII shows the results of a typical experiment (of 3) of the effect 30 mg indomethacin/kg body weight administered 30 min prior to induction of surgical shock. Indomethacin completely abrogated the shock induced decline in Fn levels which are virtually always seen at 1 to 2 hrs post surgery. (We have over 20 experiments of 2-4 rats each in which we have looked at the effect of surgery on plasma Fn levels and have seen a dip in 90+% of the rats.) The prevention of the dip by indomethacin suggests that prostaglandins do, indeed, have a negative effect on plasma Fn concentrations.

Since most drugs act on more than one system, experiments identical to those with indomethacin were done using ibuprofin as the cyclooxygenase inhibitor. The results of a typical experiment (Table VIII) are essentially identical to those seen with indomethacin. Again, the post surgical decline was eliminated. We tried to determine which of the prostaglandins are responsible for the control by repeating the above experiments using imidazole, which specifically inhibits thromboxane synthetase (Needleman et al., 1977). As can be seen from Table IX, this treatment also abrogated the shock induced dip in Fn levels, indicating that the thromboxanes are involved in regulating circulating Fn levels. In many systems, prostacyclin I_2 has effects which are antagonistic to those of thromboxane. When rats were pretreated with PGI_2, there was some indication that it prevented the decline in plasma Fn levels following surgical stress, but it also interfered with blood clotting which killed the rats before the end of the experiments (not shown).

In an effort to confirm the results from the prostaglandin inhibitor studies, rats were implanted with Alzet osmotic pumps containing TxA_2, PgI_2 or PBS. We were unable to get consistent results with these experiments probably because of variation in the length of time the prostaglandins remained active even in the presence of albumin. However, if trauma induced release of prostaglandins are indeed responsible for the concomitant decline in plasma Fn levels, it is likely that they are released very quickly after the injury. Therefore, simple subcutaneous or intraperitoneal injection of the appropriate prostaglandin(s) should have a similar effect. Rats were injected i.p. with PGI_2, TxA_2, or PBS, and blood samples were collected just prior to injection and 1, 2, 4, 6, and 8 hours following injection (Table X). Since imidazole (which specifically blocks TxA_2 synthesis) was able to abrogate the trauma induced decline in plasma Fn, it was surprising that both TxA_2 and PGI_2 induced a depression in Fn levels. However, if TxA_2 is the major prostaglandin released following surgery, blocking its release with imadazole would inhibit the trauma induced decline in plasma Fn and would make it appear that the decline was due to specific effects of TxA_2.

[redacted] these data, it appears prostaglandins, specifically TxA_2 and [redacted] PGE, act to decrease circulating Fe levels. Manipulation of prostaglandin levels may, therefore, hold promise as a means of maintaining [redacted] plasma levels of Fe for therapeutic benefit.

CONCLUSIONS

1. Fibronectin from blood binds to tissue debris and effete cells within one to two hours after the injury.

2. Macrophages at the site recognize the fibronectin on the surface of these cells and debris and phagocytize the material.

3. Fibronectin applied exogenously to the wound significantly speeds wound healing.
   a. The carrier for the fibronectin influences the efficacy.
      i. Gelfoam, Saphenous OR and polyethylene glycol are about equal.
      ii. Agarose inhibits wound healing.
   b. Treatment of the wound once a day for one or two days provides maximum improvement.

4. Plasma levels of fibronectin are controlled, at least in part, by prostaglandins.
   a. Inhibition of prostaglandin synthesis prevents surgery induced depression in Fn levels.
   b. Injection of thromboxane A_2 or prostacyclin I_2 depresses Fn levels.

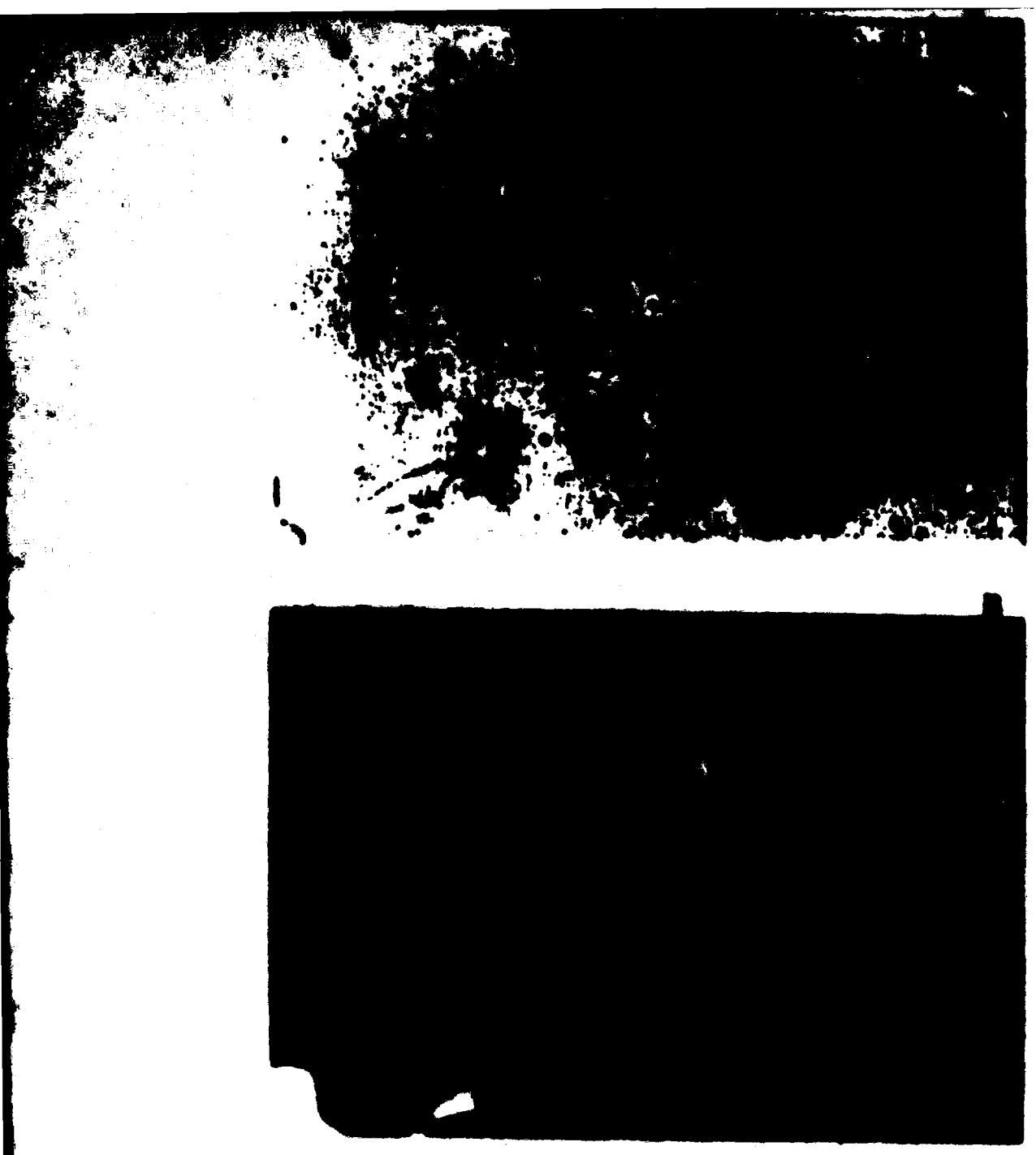


Figure 1. [illegible]

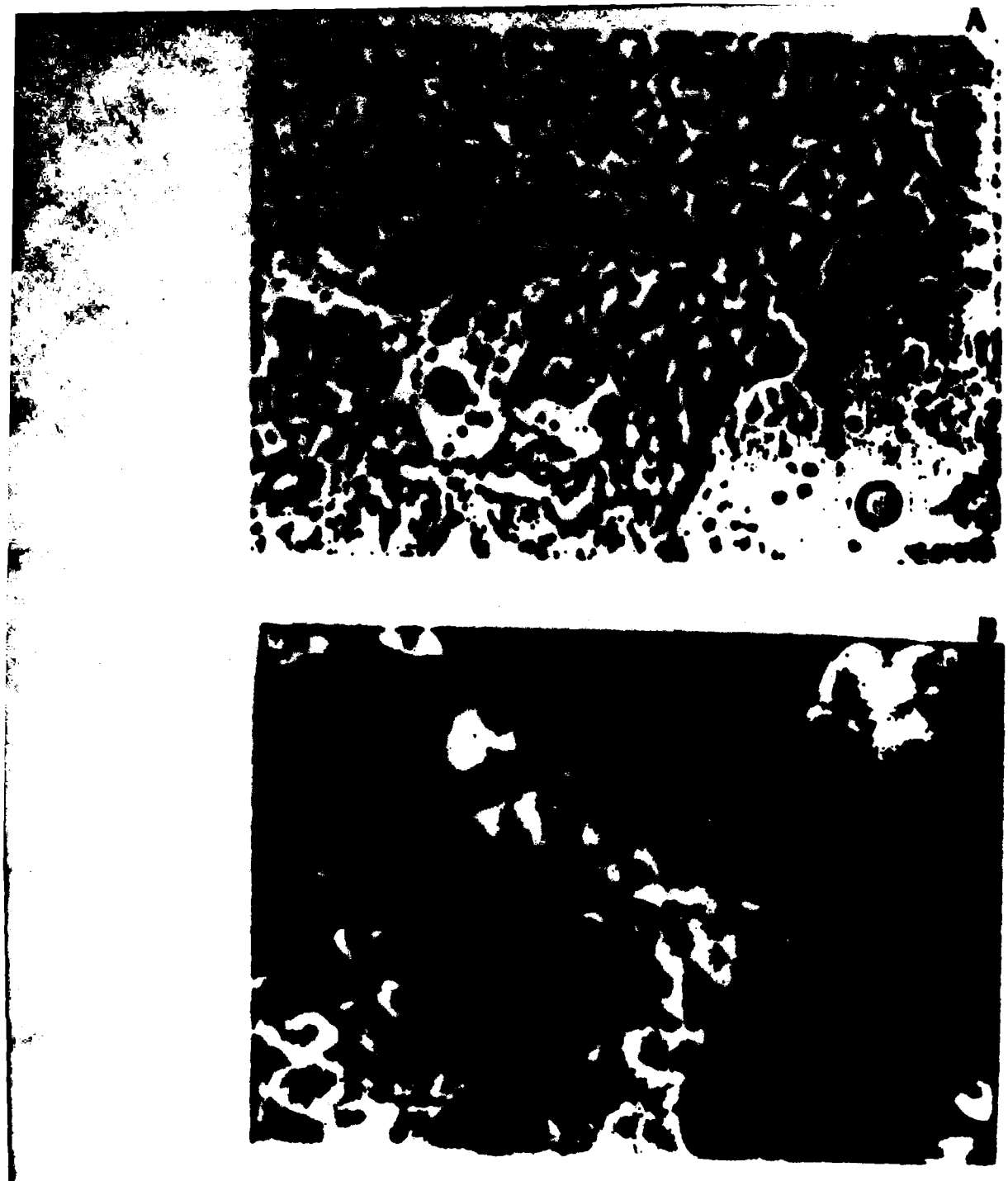
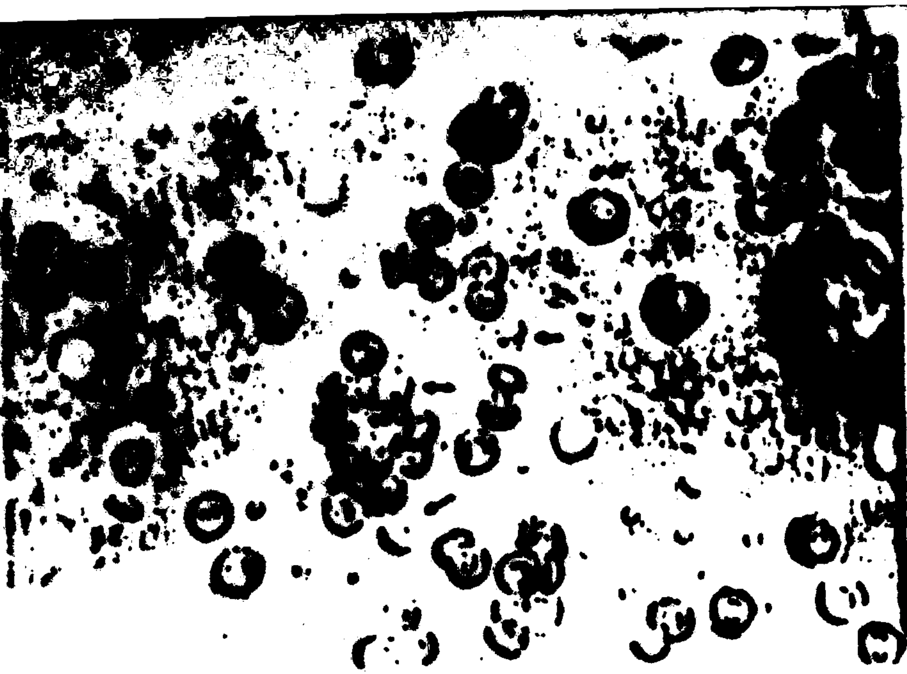


Figure 2. Fibronectin binding to ethoto cells. Conditions were identical to those in Figure 1. Again, only green fluorescence (B) was observed indicating that the binding of Fn to the damped cells was specific. Ring fluorescence indicates that only the exteriors of the cells are coated with Fn-FITC. Fluorescence of only part of the cells in the tissue is consistent with Fn-FITC binding only to damped cells.



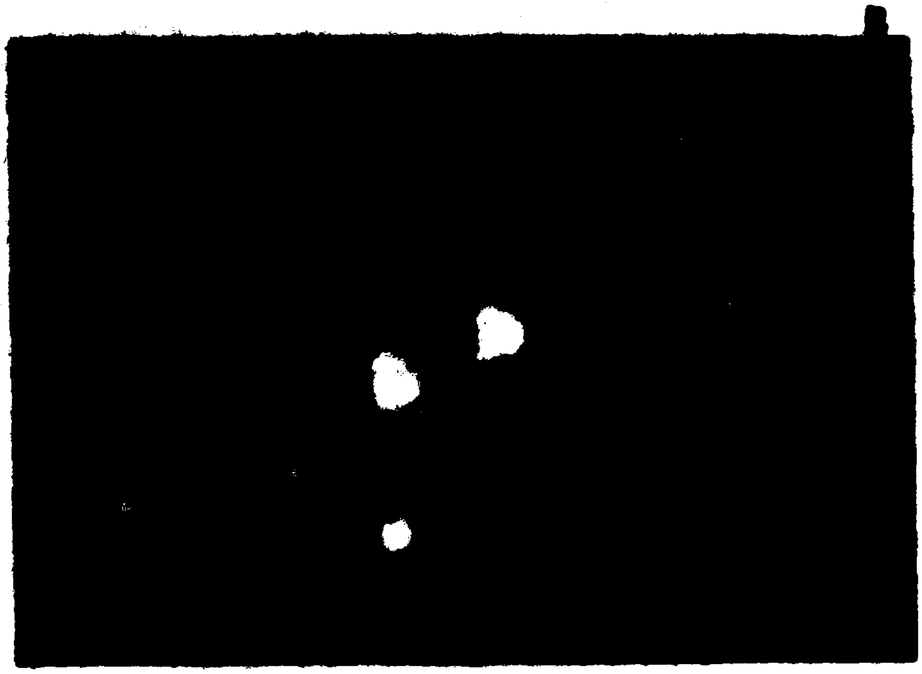


Figure [illegible]. Phagocytosis of [illegible] opsonized material by macrophages. Conditions were the same as in Figure 1 except macrophages were collected from chambers filled with Dulbecco's Minimum Essential Medium between [illegible] hrs after the abrasion was made. Internal green fluorescence indicates that the macrophages have taken up [illegible]-FITC. Lack of red ([illegible]) or yellow ([illegible]-FITC + [illegible]) shows that the [illegible] has not been taken up by phagocytosis of the tissue fluid. Arrows in A point to macrophages which are fluorescing in B.

TABLE I

Effect of Fn in Various Carriers on Wound Healing

Carrier alone or carrier (phosphate buffered saline, dimethyl sulfoxide, or Aquaphor) mixed with 500 ug Fn/ml) or Orabase mixed with 1000 ug Fn/ml was used to treat excised lesions 3 times a day. The length and breadth of the wounds were measured on the days indicated and the total area calculated using the formula for the area of an oval. For comparison, the area determined immediately after wounding was taken as 100% and the area of the wounds on succeeding days were calculated as a % of the initial area. Data from a representative experiment (of three) are expressed as the mean + SEM (n = 8).

Day	PBS	PBS-Fn	DMSO	DMSO-Fn	Aqua	Aqua-Fn	Ora	Ora-Fn
	Open wound remaining (as percent of initial wound area)							
0	100	100	100	100	100	100	100	100
2	89 + 6	86 + 4	75 + 5	80 + 8	85 + 8[A]	77 + 4[A]	74 + 3	78 + 6
5	51 + 7	45 + 2	56 + 7[A]	44 + 5[A]	78 + 5	72 + 4	57 + 4	61 + 6
7	43 + 5	35 + 2	45 + 6[A]	36 + 4[A]	68 + 5	63 + 6	31 + 5	28 + 2
9	29 + 4	27 + 2	36 + 4[C]	31 + 4[C]	53 + 3	46 + 4	12 + 2[A]	7 + 1[A]
12	21 + 2	20 + 2	21 + 2[B]	15 + 2[B]	42 + 4	38 + 4	3 + 1[B]	0 + 0[B]

A = significance of difference between pairs > 0.05 (paired Student's t test).

B = significance of difference between pairs > 0.025 (paired Student's t test).

C = significance of difference between pairs > 0.01 (paired Student's t test).

TABLE II

EFFECT OF FN IN ADDITIONAL CARRIERS ON WOUND HEALING RATE.

Standard lesions on each rat were treated twice daily with hydrophylic petrolatum (500 ug/ml), Sepharose 4B (330 ug/ml), or polyethylene glycol 20000 (250 ug/ml). The other lesion of each pair was treated with the carrier alone. The lesion treated with each combination was varied from rat to rat to correct for possible variation due to any anterior to posterior gradients. The area of the original lesion (day 0) was defined as 100%, and the size of the lesion on subsequent days was taken as the percent of the original area. Data are shown as the mean + sem of 7 observations. Values marked with * are significantly different from the wound treated with the carrier alone.

	Time of measurement (days)				
Treatment	2	4	6	9	11
	Open wound remaining (as percent of original)				
Hydrophylic Petrolatum					
Experimental	108 + 7	94 + 7	58 + 5	29 + 6	8 + 3*
Control	101 + 9	88 + 4	59 + 6	23 + 3	17 + 4
Sepharose 4B					
Experimental	89 + 7	76 + 5	51 + 5	20 + 3*	8 + 2*
Control	100 + 7	84 + 10	59 + 8	33 + 8	12 + 2
Polyethylene Glycol					
Experimental	96 + 9	81 + 5	66 + 7*	29 + 3*	13 + 3*
Control	104 + 9	90 + 12	81 + 8	43 + 7	20 + 5

TABLE III

EFFECT OF DIFFERENT TREATMENT SCHEDULES WITH FN ON WOUND HEALING.

Wounds were treated with Orabase once each day or with Orabase containing 500 ug Fn/ml once each day for the period shown. Lesions were measured immediately after wounding (100%) and on subsequent days as shown. The area of the wound was compared with the area on day 0. All treated wounds except those marked with * were significantly ($P < 0.05$) different from the untreated wounds.

	Time of measurement (days)				
Days	2	4	6	9	11
Treated	Open wound remaining (as percent of original)				
None	93 + 5	77 + 4	52 + 4	13 + 1	2.7 + 0.6
2	79 + 3	53 + 7	46 + 7*	5 + 1	0
4		64 + 3	52 + 7*	5 + 1	1.0 + 0.7
6			42 + 2	4 + 2	0.4 + 0.4
8				4 + 2	0.5 + 0.5
10				5 + 2	1.6 + 0.8*
	2	5	7	9	11
None	88 + 4	46 + 8	33 + 4	20 + 5	2.1 + 0.8
1	73 + 4	40 + 3*	20 + 4	5 + 1	0
2	71 + 6	44 + 7*	18 + 2	8 + 2	1.0 + 0.8*

TABLE IV

EFFECT OF Fn ON WOUND HEALING IN ESSENTIAL FATTY ACID DEFICIENT RATS.

Retired breeder rats were maintained on essential fatty acid deficient diets for 90 days prior to beginning the experiment. Two pairs of full dermal thickness wounds were made on the shaved dorsal surface of the anesthesized rats with a sterile paper punch. The rats were treated 3 times daily with Orabase or DMSO alone (controls) or with Orabase or DMSO containing 1 mg Fn/ml.

Day	Orabase		DMSO	
measured	Control	Fn	Control	Fn
	Open wound remaining (as percent of original)			
0	100	100	100	100
2	101 + 9	88 + 9	84 + 9	95 + 9
4	83 + 7(a)	64 + 4(a)	70 + 8	70 + 8
6	74 + 8(b)	51 + 12(b)	58 + 7	61 + 8
9	32 + 8 (a)	12 + 13(a)	20 + 4	19 + 6
12	2 + 3*	0.4 + 1~	0	0

(a) = P < 0.05 via paired Student's t test.

(b) = P < 0.001 via paired Student's t test.

*All but two of the wounds (in the same animal) were healed.

~All but one of the wounds were healed.

TABLE V

EFFECT OF SURGICAL TRAUMA ON FIBRONECTIN LEVELS IN ESSENTIAL FATTY ACID DEFICIENT RATS

Surgical shock was induced as in Table V in young adult rats which had been maintained on an essential fatty acid free diet for 8 weeks prior to the experiment. Control rats were maintained on Purina Lab Blox ad libitum. Blood samples were collected and analyzed as for Table V above. Results of a typical experiment are shown and expressed as the mean ± SEM of quadruplicate determinations.

Treatment	Time after incision and intestinal manipulation						
	0 hr	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
Control 1	412 ± 10	432 ± 13	430 ± 7	416 ± 16	430 ± 5	448 ± 9	530 ± 3
Control 2	482 ± 11	481 ± 11	508 ± 10	461 ± 10	445 ± 10	448 ± 6	554 ± 4
Trauma 1	432 ± 0	432 ± 4	448 ± 3	429 ± 3	406 ± 4	396 ± 2	Dead
Trauma 2	506 ± 13	506 ± 13	496 ± 11	493 ± 1	482 ± 14	504 ± 16	532 ± 6

TABLE VI

EFFECT OF SURGICAL TRUAMA ON PLASMA FIBRONECTIN LEVELS

Normal young adult rats were anesthesized with ether, and a 2-3 cm midline laporatomy produced. About 5 cm of small intestine was exteriorized, kneeded gently for about 30 sec, and replaced in the abdominal cavity. The muscle layer was sutured with 4.0 silk, and the skin was closed with wound clips. Blood samples were taken at the times indicated, and Fn concentration in the plasma determined using an ELISA based competative inhibition assay. Results of a typical experiment are shown and expressed as the mean ± SEM of quadruplicate determinations.

Treatment	Time after incision and intestinal manipulation						
	0 hr	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
Control 1	318 ± 7	338 ± 18	340 ± 22	344 ± 8	360 ± 0	404 ± 4	389 ± 18
Control 2	354 ± 22	376 ± 24	386 ± 22	376 ± 32	340 ± 10	368 ± 8	301 ± 10
Trauma 1	248 ± 8	249 ± 11	212 ± 12	258 ± 10	268 ± 7	282 ± 7	328 ± 16
Trauma 2	304 ± 12	250 ± 16	281 ± 2	285 ± 7	276 ± 14	274 ± 10	321 ± 14

TABLE VII

EFFECT OF SURGICAL TRAUMA ON FIBRONECTIN LEVELS IN RATS TREATED WITH INDOMETHACIN

Surgical trauma was induced as in Table V in young adult rats which had been treated 30 min previously by an i.p. injection with 30 mg indomethacin (indo)/kg body weight. Control animals were injected with a similar volume of phosphate buffered saline (PBS). Blood samples were collected and analyzed as for Table V above. Results of a typical experiment are shown and expressed as the mean ± SEM of quadruplicate determinations.

Treatment	Time after incision and intestinal manipulation						
	0 hr	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
PBS 1	314 ± 6	264 ± 10	284 ± 8	294 ± 2	318 ± 12	346 ± 32	dead
PBS 2	346 ± 32	356 ± 18	296 ± 3	288 ± 7	330 ± 10	296 ± 0	328 ± 12
Indo 1	356 ± 12	356 ± 2	356 ± 7	344 ± 12	328 ± 7	336 ± 14	378 ± 2
Indo 2	324 ± 18	332 ± 7	296 ± 16	360 ± 4	372 ± 14	370 ± 8	292 ± 8

TABLE VIII

EFFECT OF SURGICAL TRAUMA ON FIBRONECTIN LEVELS OF RATS TREATED WITH IBUPROFEN

Surgical shock was induced as in Table V in young adult rats which had been treated 30 min previously by an i.p. injection with 30 mg ibuprofin (Ibp)/kg body weight. Control animals were injected with a similar volume of phosphate buffered saline (PBS). Blood samples were collected and analyzed as for Table V above. Results of a typical experiment are shown and expressed as the mean ± SEM of quadruplicate determinations.

Treatment	Time after incision and intestinal manipulation						
	0 hr	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
PBS	476 ± 15	408 ± 6	449 ± 18	464 ± 8	423 ± 14	460 ± 11	537 ± 30
PBS	456 ± 8	417 ± 4	457 ± 18	464 ± 22	493 ± 26	485 ± 14	572 ± 21
Ibp 1	560 ± 18	576 ± 20	546 ± 21	498 ± 13	616 ± 11	612 ± 19	616 ± 11
Ibp 2	424 ± 12	446 ± 26	461 ± 18	552 ± 5	572 ± 32	564 ± 4	565 ± 21
Ibp 3	488 ± 13	478 ± 17	513 ± 17	484 ± 16	497 ± 10	464 ± 5	545 ± 14
Ibp 4	474 ± 10	522 ± 10	456 ± 9	506 ± 10	448 ± 10	481 ± 10	604 ± 11
Ibp 5	490 ± 3	504 ± 14	488 ± 16	460 ± 6	464 ± 14	508 ± 18	569 ± 25

TABLE IX

EFFECT OF SURGICAL TRAUMA ON FIBRONECTIN LEVELS OF RATS TREATED WITH IMADAZOLE

Surgical trauma was induced as in Table V in young adult rats which had been treated 30 min previously by an i.p. injection with 30 mg imadazole (Idz)/kg body weight. Control animals were injected with a similar volume of phosphate buffered saline (PBS). Blood samples were collected and analyzed as for Table V above. Results of a typical experiment are shown and expressed as the mean ± SEM of quadruplicate determinations.

Treatment	Time after incision and intestinal manipulation						
	0 hr	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
PBS 1	396 ± 15	297 ± 6	341 ± 6	364 ± 17	392 ± 6	395 ± 2	418 ± 2
PBS 2	384 ± 3	300 ± 13	300 ± 14	346 ± 17	364 ± 20	380 ± 14	480 ± 0
Idz 1	416 ± 16	376 ± 6	384 ± 7	416 ± 15	416 ± 16	368 ± 8	474 ± 2
Idz 2	405 ± 16	394 ± 4	404 ± 12	405 ± 12	408 ± 11	429 ± 20	dead

[redacted] X

[redacted] OF INCREASED [redacted] ON PLASMA FIBRONECTIN LEVELS

Sprague-Dawley rats (weighing approximately 300 gm each) were injected i.p. with 100 ug of a [redacted]-PBS solution (controls) or with thromboxane A2 in [redacted]-PBS or with prostacyclin I2 in [redacted]-PBS. At the times indicated the rats were given light ether anesthesia and two to three ml of blood taken via heart puncture. Fn concentrations were determined with the competitive inhibition assay. Results are expressed as mean ug Fn/ml blood of three values ± sem and as the mean percentage of the control value ± sem.

Treatment	Time after injection					
	0 hr	1 hr	2 hr	4 hr	6 hr	8 hr
Control	420 ± 24	469 ± 21	408 ± 16	380 ± 26	376 ± 32	372 ± 68(a)
%	100	106 ± 6	96 ± 2	91 ± 3	88 ± 5	87 ± 8(a)
TxA2	461 ± 27	365 ± 30*	384 ± 24	340 ± 16*	376(b)	328(b)
%	100	79 ± 2	86 ± 5	76 ± 8	82(b)	72(b)
PgI2	400 ± 14	387 ± 41	364 ± 28	336 ± 12	386 ± 4(a)	364 ± 32(a)
%	100	79 ± 7	77 ± 9	72 ± 6	80 ± 9(a)	78 ± 12(a)

* = significantly different from preinjection levels ($P < .05$ as determined by a paired Student's t test).

a = one rat died so only 2 values were used for the mean.

b = two rats died so a single value is given.

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