determining the peak position on these islands (*Lantana*, ripe *Rhynchosia*, and *Opuntia*). On Daphne 42 percent of *G. fortis* that tried to crack *Bursera* stones were successful (*11*), whereas on Pinta 77 percent were successful (*25*).
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21 December 1983; accepted 18 October 1984

Regulation of Extravascular Coagulation by Microvascular Permeability

Abstract. *Extravascular coagulation is a prominent feature of such important pathological processes as cellular immunity and neoplasia and has been thought to result from procoagulants associated with the inflammatory or tumor cells peculiar to these entities. It was found that increased microvascular permeability alone is sufficient to induce equivalent extravascular coagulation in several normal tissues. The results indicate that saturating levels of procoagulant are present even in normal tissues and that microvascular permeability is a rate-limiting step in extravascular coagulation.*

Interest in the clotting system has focused on intravascular coagulation—the thrombotic and embolic processes that are intimately associated with atherosclerosis, myocardial infarction, and cerebrovascular disease (*1*). However, extravascular coagulation is a prominent feature of many important pathological entities, including cell-mediated immunity and neoplasia (*2*). Thus, fibrin is deposited locally in delayed hypersensitivity reactions and in solid tumors. This fibrin is derived from plasma fibrinogen that has extravasated from blood vessels, clotted, and become cross-linked in the tissue space (*3*). The biological significance of extravascular coagulation in these and other disease processes is only now beginning to be investigated. It is known that extravascular deposits of fibrin, by forming a water-trapping gel, cause the induration characteristic of delayed hypersensitivity and contribute to brain damage in autoimmune encephalitis (*4*). In addition, extravascular clotting and associated fibrinolysis have been implicated in depressing the immune response, in enhanced vessel permeability, and in tumor growth, angiogenesis, desmoplasia, and metastasis (*2*, *5*).

How is extravascular coagulation regulated? Much attention has been devoted to the characterization of cellular procoagulants that can initiate or promote clotting, particularly macrophage and tumor cell membrane-associated molecules that act at various steps in the coagulation pathway (*2*, *6*). However, one potential control point that has received little or no attention is the availability in the extravascular space of plasma clotting factors, particularly fibrinogen. Under conditions of normal low vascular permeability, levels of extravascular fibrinogen are negligible, bordering on the limit of detection (*3*). If significant extravascular fibrin is to be deposited, therefore, tissue fibrinogen and probably other plasma clotting factors must first be increased. Increased vascular permeability to plasma proteins regularly parallels extravascular coagulation in both cellular immunity and malignant tumors (*2*).

We therefore investigated the relation of blood vessel permeability to extravascular coagulation in several normal tissues. Adult Hartley guinea pigs were injected intravenously with ^{125}I-labeled guinea pig fibrinogen (GPF) and immediately thereafter were challenged locally with histamine, bradykinin, or a tumor-secreted vascular permeability mediator (VPM) (*7*), all agents that transiently increase venule permeability. Twenty minutes later the quantity and nature of locally accumulated [^{125}I]GPF were determined (*3*, *8*, *9*).

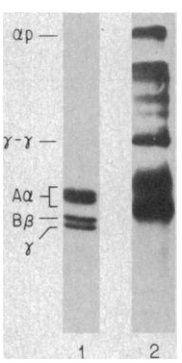

Fig. 1. Autoradiogram of reduced sodium dodecyl sulfate–polyacrylamide gel of [^{125}I]GPF (track 1) and of urea residue from skin taken 20 minutes after injection of histamine (track 2). Injected guinea pig fibrinogen shows, as expected, Aα (doublet), Bβ, and γ chains. [^{125}I]GPF circulating in plasma showed an identical pattern. Prominent bands corresponding to γ-chain dimers (γ-γ; molecular weight, ~93K), and high molecular weight α-chain polymers (αp; >200K), are present in gels from the histamine skin test site—indicative of cross-linked fibrin. A variety of uncharacterized fibrinogen-fibrin breakdown products are also represented in the latter gel.

Intradermal injection of histamine led to a dose-dependent increase in [^{125}I]GPF accumulation greatly exceeding that of uninjected or saline-injected skin (Table 1). Thus 1 μg of histamine led to the accumulation of 118 μl of platelet poor plasma (PPP), equivalent to 394 μg of fibrinogen, per gram of skin. Moreover, most [^{125}I]GPF extravasated at histamine injection sites was not extractable with aqueous buffer or with 3*M* urea and thus had acquired properties of cross-linked fibrin (*2*, *3*, *9*). Neither [^{125}I]GPF accumulation nor clotting was increased further when guinea pig brain tissue factor (*10*) or thrombin (0.2 U) was included in the histamine injections, suggesting that the amount of procoagulant activity present in normal skin was sufficient and not rate-limiting. Similar results were obtained when bradykinin or VPM was used to enhance skin vessel permeability or when the effects of local histamine were studied in the eye (Table 1) or the peritoneal wall.

Local anticoagulation achieved by including clotting inhibitors in histamine-containing eye drops (Table 1) or in injections of the skin with histamine strikingly inhibited the amount and fraction of extravasated [^{125}I]GPF that was insoluble in urea. Similar results were obtained when animals were treated systemically with heparin.

Conclusive evidence for the cross-linked nature of the fibrin deposited in histamine skin test sites was obtained from autoradiograms of sodium dodecyl sulfate–polyacrylamide gels subjected to electrophoresis (Fig. 1) (*11*). The autoradiograms showed extensive γ-γ dimerization and α-chain polymerization, characteristic of cross-linked fibrin (*1*, *3*, *9*). Thus the fibrin deposited in skin as the result of locally increased microvascular permeability had the same biochemical characteristics as that found in solid tumors and in skin undergoing delayed hypersensitivity reactions (*3*).

The extravascular localization of tissue fibrin was further defined by immunohistochemistry and electron microscopy of histamine skin test sites (Fig. 2). Fibrin was concentrated in focal deposits centered about individual fixed connective tissue cells, fibrocytes, and histiocytes. The fibrin, which transmis-

sion electron microscopy showed to have a characteristic banding pattern, extended from these foci, forming a three-dimensional gel meshwork between dermal collagen bundles. Much smaller amounts of fibrin were observed in saline test sites, and none was seen in uninjected skin.

Although all the animals received anticoagulants intravenously before being killed and blood samples taken at death gave infinite clotting times, we had no independent way of determining whether these anticoagulants entered the tissues in a quantity sufficient to interfere with extravascular coagulation. Therefore it could be argued that the fibrin deposited in histamine test sites resulted from clotting that occurred only in the final moments of the experiment, as the result of procoagulants released in association with the trauma of tissue dissection. However, we were able to exclude this possibility in the eye. Anticoagulant drops, which effectively inhibited extravascular clotting when administered concurrently with histamine 20 minutes before tissue harvest, did not reduce the extent of extravascular clotting when applied immediately before tissue harvest, 20 minutes after the administration of a histamine-containing eye drop.

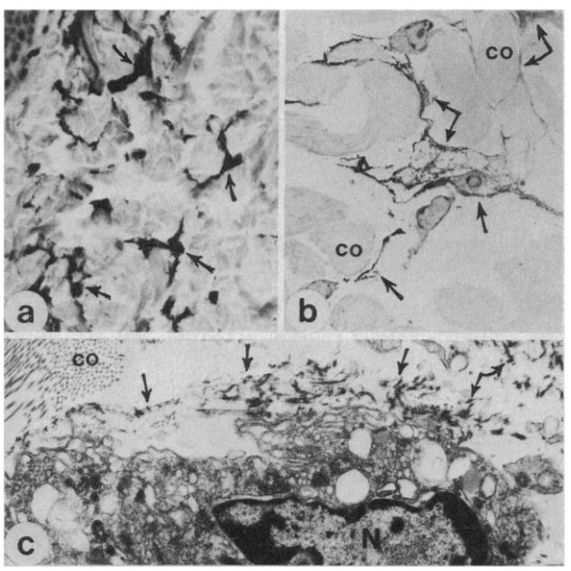

Fig. 2. Fibrin distribution in skin taken 20 minutes after histamine injection, as revealed by light microscopy by the immunoperoxidase technique in paraffin (a), in 1-μm Epon-embedded sections (b), and by transmission electron microscopy (c). Note the close association of fibrin (arrows) with fixed tissue cells and the extension between dermal collagen bundles. Abbreviations: co, collagen and N, nucleus of fixed tissue histiocyte. Magnifications: (a) ×100, (b) ×900, and (c) ×7000.

Table 1. Accumulation of total and urea-insoluble (cross-linked) [^{125}I]GPF in normal guinea pig tissues as a function of microvascular permeability. The animals were given intravenous injections of [^{125}I]GPF (5×10^6 count/min) and were then administered test and control substances. For skin measurements, intradermal injections of 0.2 ml in saline were made into depilated flank skin. For eye studies, the animals were lightly anesthetized with ketamine and a single drop of 50 μl was applied to the cornea. The animals were killed 20 minutes later and tissues were harvested (*3*, *8*).

Test substance	Number*	[^{125}I]GPF		
		Total (μl/g)†	Urea-insoluble (μl/g)†	Urea-insoluble (percent)
		Skin		
Histamine				
0.1 μg	4	63 ± 11	35 ± 10	56
1.0 μg	24	118 ± 8	85 ± 7	72
10 μg	4	391 ± 35	303 ± 33	77
Bradykinin (0.2 μg)	8	75 ± 10	55 ± 9	73
VPM (2.8 μg)	4	336 ± 30	247 ± 27	74
Saline	20	17 ± 2	5 ± 1	29
No injection	17	10 ± 1	2 ± 1	20
		Extraocular muscles		
Histamine (50 μg)	5	390 ± 43	287 ± 48	74
Saline	8	41 ± 13	9 ± 2	22
Histamine (50 μg) + anticoagulant‡	6	229 ± 83	53 ± 19	23
Anticoagulant alone‡	4	53 ± 12	7 ± 2	13

*Number of separate skin test sites or eyes. †Expressed as microliters of PPP (means ± standard errors) (*8*). One microliter of guinea pig PPP contains 3.34 μg of fibrinogen. In every case, total GPF, urea-insoluble GPF, and the percentage of urea-insoluble GPF in histamine, bradykinin, and VPM injection sites were significantly different from saline or uninjected skin sites ($P < 0.01$, Duncan's test for multiple comparisons) (*15*). ‡Anticoagulant in each eye drop contained 4 U of hirudin (Sigma), 20 U of heparin, and 40 μg of soybean trypsin inhibitor in saline.

It was also possible that the procoagulant we observed was not active in undisturbed tissue, but rather was expressed only after local trauma, such as the minor tissue injury that inevitably accompanies intradermal injections. Indeed, saline-injected skin sites exhibited increased extravasation of [^{125}I]GPF and clotting compared to uninjected skin (Table 1). However, application of a single histamine-containing eye drop led to both increased vessel permeability and extravascular clotting in the extraocular muscles (Table 1) and the limbus. Similarly, substantial accumulation of fibrin was observed in the diaphragm and the peritoneal wall, tissues remote from a site of intraperitoneal histamine injection. Thus we conclude the procoagulant activity is expressed in normal tissues independent of tissue injury. However, our results do not exclude the possibility that the procoagulant of normal tissues may normally be cryptic and becomes expressed only on activation by plasma components leaked into tissues as the result of increased microvascular permeability. This possibility could account for the increased fraction of extravasated [^{125}I]GPF that is rendered urea-insoluble when vessels are rendered hyperpermeable (Table 1).

In summary, we found that most of the plasma fibrinogen extravasated from hyperpermeable blood vessels in several otherwise normal tissues was rapidly clotted and cross-linked. Extravascular clotting occurred independently of the agent used to enhance microvascular permeability and was inhibited by local or systemic anticoagulants. Inflammatory cells and tumor cells were absent; therefore neither was required for the clotting observed. Rather, fibrin deposits were associated anatomically with fixed tissue fibrocytes and histiocytes, suggesting that the procoagulant of normal tissues is associated with these cells. We anticipate that this cell-associated procoagulant activity includes tissue factor and prothrombinase-generating activities (*2*, *13*).

That extravasated plasma clots in normal tissues in the absence of cell injury indicates that microvascular permeability provides an important regulatory mechanism for the control of extravascular coagulation. Whether other plasma inflammatory pathways, such as the kinin and complement systems, are similarly activated by increased vascular permeability remains to be determined. In-

creased microvascular permeability is also a regular feature of cellular immunity and neoplasia and is mediated by specific permeability factors secreted by activated lymphocytes (*14*) and tumor cells (*7*), respectively. We suggest, therefore, that increased vascular permeability, rather than special procoagulants associated with macrophages or tumor cells, also accounts for the substantial extravascular deposits of fibrin regularly found in cellular immunity and in solid tumors.

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8. Guinea pig fibrinogen was purified, labeled with ^{125}I (*3*), and injected intravenously into each animal. Test and control substances were then administered. Twenty minutes later, guinea pigs received intravenously 0.5 ml of an anticoagulant-antifibrinolytic mixture containing heparin (1000 U), hirudin (100 U) or D-phenylalanyl-L-propyl-L-arginine chloromethyl ketone (250 μg), ε-aminocaproic acid (EACA) (25 mg), and Trasylol (700 U) in 0.15*M* NaCl. One minute later the animals were anesthetized with ether and exsanguinated. Blood samples were centrifuged (10,000*g* for 20 minutes) to prepare PPP for counting of radioactivity. Test and control tissues were dissected into tared tubes containing 2 ml of ice-cold 0.01*M* phosphate buffer (*p*H 7.5) supplemented with heparin (10 U/ml), hirudin (2 U/ml), EDTA (2 mg/ml), 0.1*M* EACA, 2 m*M* phenylmethylsulfonyl fluoride, 2 m*M* iodoacetate, and 2 m*M* *N*-ethylmaleimide (*3*). Test and control tissues were weighed wet. Tissue radioactivity (counts per minute per gram) was divided by that in PPP (counts per minute per microliter of plasma) to express accumulation of [^{125}I]GPF as microliters of PPP that entered each gram of test or control tissue. Microliters of PPP can be converted to fibrinogen equivalents (micrograms) by multiplying by 3.34 (*3*). To characterize the [^{125}I]GPF deposited, tissue samples were minced and extracted first with tissue buffer (to solubilize fibrinogen, fibrin monomer, and water-soluble fibrin degradation products) and then with 3*M* urea (to solubilize non–cross-linked fibrin) (*9*). Radioactivity remaining in the urea-insoluble residue, largely representing cross-linked fibrin, was counted to calculate cross-linked fibrin accumulation.
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7 September 1984; accepted 19 November 1984

Genetic Consequences of Mate Choice: A Quantitative Genetic Method for Testing Sexual Selection Theory

Abstract. *To investigate whether female mate choice could be directed at male genetic quality, male chemical signals and progeny fitness were studied in the red flour beetle (*Tribolium castaneum*). Differences among males in the attractiveness of their pheromone to females were statistically significant. Developmental time of progeny was significantly heritable, indicating that some males have "good genes" for this trait. There was no statistically significant correlation between progeny fitness and male attractiveness. These results do not support the hypothesis that in this species the evolution of female preferences for male pheromone is adaptive.*

In species in which males provide neither resources nor parental care as part of reproduction, the evolution of sexual dimorphism as a result of female mate choice has been explained by two opposing models. The models are similar in that the evolution of conspicuous male displays is seen to result from selection due to female choice; they differ in their explanation of the evolution of female choice. Fisher and others (*1*) described a process in which extreme female mating preferences evolve as a correlated response to sexual selection on the male display, not as a consequence of increased fitness of discriminating females. This process originates when preference by some females for a particular trait in some males leads to a genetic covariance between the loci controlling the female trait (choice) and the male trait (display) as a result of assortative mating for these traits. A genetic covariance permits these traits to evolve because the male offspring of discriminating females bear a trait that makes them more attractive and female offspring bear a trait making them more discriminating.

Contrasting models emphasize the adaptive nature of female choice: females choose males because traits in the male display are correlated with components of male fitness that are under natural as opposed to sexual selection (*2*). That is, females mate with males possessing "good genes" and thereby produce progeny that are more viable than those sired by less conspicuous males. A crucial issue in these models is whether there are "good genes" that are correlated with traits detectable by females (*3*). I describe an experiment in which quantitative genetic techniques were used to measure progeny fitness of males that differ in their attractiveness to females.

First, male attractiveness is evaluated through behavioral tests with conspecific females. Second, the genetic quality of the same males is evaluated by quantitative genetic techniques. Males are considered to differ in genetic quality if components of fitness measured in their progeny have significant (narrow sense) heritability. Third, the extent to which females recognize and prefer males bearing good genes is evaluated by computing the correlation between attractiveness of sires to females and the fitness of progeny of these sires. This procedure is applied to an analysis of male attractiveness and genetic quality in the red flour beetle (*Tribolium castaneum*).

Attractiveness of males to females. Adult male flour beetles at low densities in high quality habitat emit a volatile pheromone that attracts conspecific females and males (*4*). These general attractant characteristics make the signal analogous to the advertisement calls and calling songs of anuran amphibians and crickets (*5*). The pheromone gives females an opportunity to make a general decision about which male to approach. Once near a male, a female apparently cannot exert mate choice: males attempt to copulate with any discontinuity in the flour, and the flour medium appears to be too soft for a female to dislodge a male.

The attractiveness of each of 16 males was determined empirically through behavioral tests of their pheromone in a two-choice pitfall trap apparatus (*6*) in which each male's pheromone was com-