objects. However, no data from either recording or lesion studies define the possible role of the retinal projection to the anterior thalamic neuropil in barrier avoidance.

There is already some evidence that functions of retinothalamic and retinotectal fibers can be dissociated in the shark, where a simple pattern discrimination suffers more from removal of the telencephalon (*7*) than from tectal ablation (*8*). In these demonstrations the discrimination stimuli were vertical and horizontal stripes—stimuli quite comparable to the grid barrier detected by the tectally injured frog. In fact, normal frogs spontaneously discriminate between vertical and horizontal grids and between vertical and horizontal apertures (*9*). While comparisons of visual mechanisms in the frog and in mammals are still tenuous, my distinction between motion detection by the tectum and stationary edge detection by the thalamus in frogs does parallel the pervasive distinction between sensitivity to motion and sensitivity to edge orientation in the tectal and thalamocortical systems in mammals. In support of this hypothesis, Casagrande (*10*) observes that tree shrews with tectal ablations also fail to localize food objects but, like frogs, are surprisingly successful in negotiating stationary barriers and darting through holes. These new data on sharks, frogs, and tree shrews support the view that the anatomical similarities in retinofugal patterns from fish to primate (*11*) provide the foundations for a basic set of visual functions which have been elaborated but not reconstructed during evolution.

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Enhanced Protein Adsorption at the Solid-Solution Interface: Dependence on Surface Charge

Abstract. *By the use of infrared internal reflection spectroelectrochemistry, it has been possible to observe an enhanced adsorption of porcine fibrinogen onto a germanium surface at potentials more positive than −200 millivolts relative to a saturated calomel electrode. The enhanced adsorption was observed directly at the interface between the solid and the aqueous solution.*

Several investigators (*1*) have demonstrated that the formation of a protein film precedes platelet adhesion during thrombogenesis on foreign surfaces. Baier and his co-workers (*2*) contend that the protein layer transmits the particular characteristics of a given surface into the blood, whereas other investigators (*3*) suggest that the free energy of the surface is responsible for its thrombogenicity. Sawyer and his co-workers (*4*) have observed that surfaces with a uniform positive charge attract the negatively charged blood platelets and proteins. Many empirical in vivo studies have been carried out in attempts to clarify this phenomenon, with substantive data (*5*) indicating that the surface charge does play an important role in formation of thrombus.

We report evidence here in support of the theory that a relatively positive charge at the surface of a germanium electrode substantially enhances the extent of adsorption of fibrinogen from aqueous saline solution. We measured the adsorption of fibrinogen in vitro by infrared internal reflection spectroscopy, using a germanium prism as both the electrode and the internal reflection element.

Internal reflection spectroscopy has been discussed in detail by Harrick (*6*), and the technique of simultaneous electrochemistry and internal reflection spectroscopy has been described in several papers (*7*, *8*). In this study we employed a Perkin-Elmer model 180 infrared spectrophotometer, with the manufacturer's internal reflection accessory. Germanium prisms (52.5 by 20 by 2 mm and 52.5 by 20 by 1 mm, Harrick Scientific Corporation) provided, respectively, 25 and 50 internal reflections at an angle of incidence of 45°. Because of cell geometry, only 11 and 22, respectively, of these reflections were actually at the germanium-solution interface. We constructed a two-compartment Teflon electrolysis cell similar to that described by Tallant and Evans (*8*), with the following modifications: (i) the counter electrode was a 10-cm platinum coil; (ii) electrical contact with the germanium prism was obtained by clamping a piece of aluminum foil to the entire "back" face of the prism (this was done to eliminate the possibility of variations in the potential from the edge to the center of the electrode); and (iii) the reference electrode was a conventional saturated calomel electrode (SCE), connected to the working electrode compartment by a 16-gauge Teflon catheter filled with saline agar gel. A potentiostat (Wenking model 70TS1) was used to maintain the desired potential, which was measured with an electrometer (Keithley model 602).

The germanium prism was cleaned before use by rinsing with concentrated HF, and then it was placed in a radio-frequency gas discharge for approximately 30 minutes. The solutions were prepared from doubly distilled, deionized water and reagent grade NaCl. Porcine fibrinogen (60 percent clottable) was obtained from Miles Laboratories and kept at 5°C until used. For the experiments reported below the solutions used consisted of 0.15*M* NaCl and 0.4 percent (by weight) fibrinogen in 0.15*M* NaCl solution. All potentials are relative to those of a SCE unless otherwise stated.

Figure 1 reproduces 14 infrared internal reflection spectra. The spectra are shown in the linear absorbance mode, and in each case the spectrum was manually displaced upward without changing the relative magnitude of the absorbance value. The peaks at 1205 and 1150 cm^{-1} are due to the Teflon, and the little blip at 1486 cm^{-1} is due to a filter change. In Fig. 1 the amide I band at 1650 cm^{-1} is naturally obscured by the water peak at 1640 cm^{-1} and so the amide II band at 1540 cm^{-1} is the "indicator" peak. Other protein absorption bands occur from 1400 to 1000 cm^{-1}, but they are quite diffuse.

The 14 spectra of Fig. 1 may be described as follows. The bottom spectrum, spectrum 1, is the germanium-cell base line. Spectrum 2 was taken

while the germanium was held at its initial rest potential, − 640 mv (relative to a SCE), in contact with the 0.4 percent fibrinogen solution in 0.15*M* NaCl. Some spontaneous adsorption does take place at this potential, but it is not observable in Fig. 1. This initial adsorption is examined later, in the discussion of Fig. 2. Stepping the potential to more cathodic values (−840, −1080, and −1200 mv) in a separate experiment resulted in no change from spectrum 2. Proceeding in the more positive direction, spectra 3, 4, 5, 6, and 7 were obtained at potentials of −500, −400, −300, −250, and −200 mv, respectively. In spectrum 8, at −175 mv, a change in slope is observed in the region of the amide II band (1540 cm^{-1}). This change in slope is recognizable in spectra 9, 10, 11, and 12, taken at potentials of −150, −125, −100, and −50 mv, respectively. In spectra 13 and 14, taken at potentials of 0 and +50 mv, respectively, the amide II peak is distinctly visible in the 1540 cm^{-1} region. The enhanced adsorption observed at potentials from −200 to +50 mv was not reversible. Stepping the potential back to −400 mv, which was the new germanium rest potential, caused no change in the magnitude of the amide II peak.

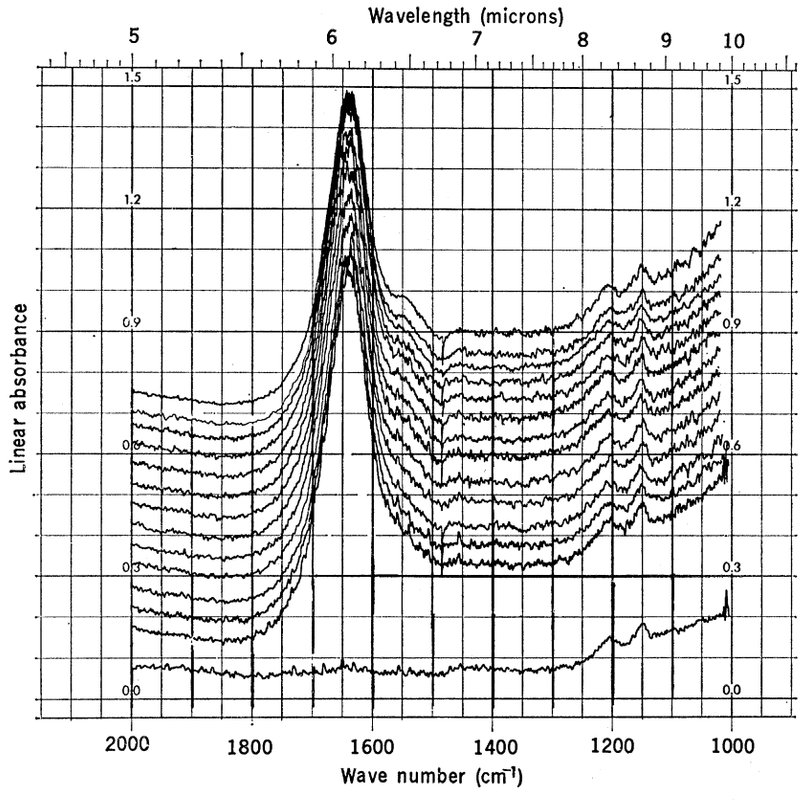

Fig. 1. Internal reflection spectra of enhanced fibrinogen adsorption at the solid-liquid interface as a function of potential: parallel polarized incident energy; 11 reflections at the solid-liquid interface; 45° angle of incidence. See the text for a discussion of the spectra.

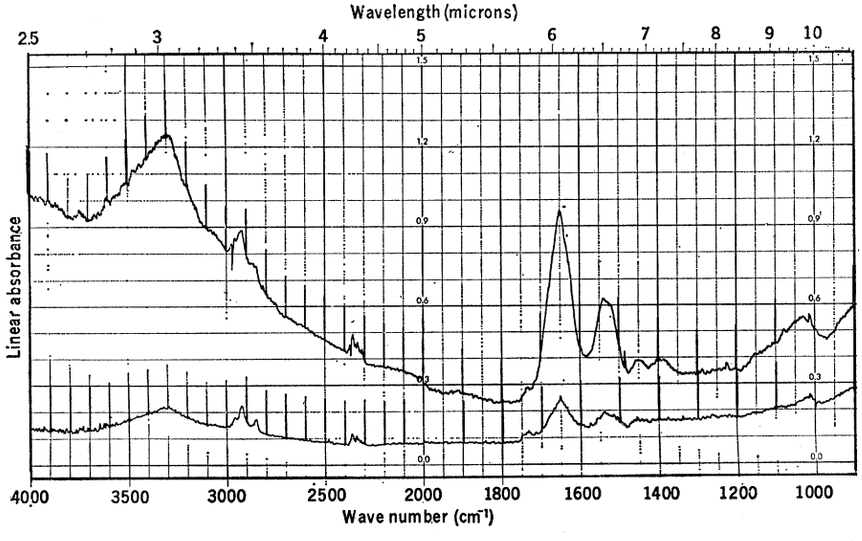

Fig. 2. (Bottom spectrum) "Spontaneous" adsorption of fibrinogen at the germanium rest potential (22 reflections). (Top spectrum) Enhanced adsorption at +50 mv (relative to a SCE) (11 reflections, 2× scale expansion). Both films were air-dried; parallel polarized incident energy; 45° angle of incidence.

The current densities involved were not large, ranging from about 2 $\mu a/cm^2$ at − 500 mv to 100 $\mu a/cm^2$ at +50 mv. An identical experiment on a "blank" 0.15*M* NaCl solution indicated that the appearance of the amide II band is not an artifact.

Figure 2 illustrates the extent to which the fibrinogen will adsorb spontaneously at the initial germanium rest potential (about −600 mv). A clean, 50-reflection prism was mounted in the cell, the cell filled with 0.15*M* NaCl, and the potential measured. The "blank" solution was then flushed out with five cell volumes of 0.4 percent fibrinogen solution (in 0.15*M* NaCl) and the potential measured again. No change was observed in the rest potential. Finally, the fibrinogen solution was flushed out with about 50 cell volumes of distilled water. The prism was removed and washed in flowing distilled water. The bottom spectrum of Fig. 2 illustrates the amount of fibrinogen that had been "spontaneously" adsorbed after the prism had been allowed to air-dry. The upper spectrum is a 2× scale expansion (because this prism had only one half the number of reflections as the prism used for the lower spectrum) of the air-dried protein film deposited in the experiment shown in Fig. 1. The electrochemically induced film is thicker than the depth of penetration of the evanescent wave (*6*), so the relative intensities of Fig. 2 are somewhat misleading. Transmission spectra of the air-dried films indicate that the electrochemically induced film is 15 to 20 times thicker than the spontaneously adsorbed film.

It is necessary to relate the potentials of enhanced adsorption (> −200

mv) and the rest potentials for the germanium (−500 to −650 mv) to the point of zero charge (PZC) for germanium. The PZC for germanium (*p* and *n* types) has been reported for 0.1*N* HCl as −900 mv (relative to a SCE), on the basis of capacitance determinations (*9*). Both the rest potential and the potential for enhanced adsorption are several hundreds of millivolts more positive than the PZC.

We conclude that the surface charge does indeed play a strong role in enhancing fibrinogen adsorption from saline solution, with a potential dependence very similar to that observed by Sawyer and his co-workers (*4*). However, this conclusion does not imply that the PZC is the same in our experiments as in those of Sawyer and his co-workers. The technique of infrared internal reflection spectroelectrochemistry should be extremely useful in continued studies of biological adhesion phenomena. The technique is not limited to germanium but can be extended to thin films such as carbon, platinum, copper, iron, and aluminum, by means of vacuum deposition of these materials onto the surfaces of internal reflection elements (*10*).

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Breast Cancer: Induction of Differentiation by Embryonic Tissue

Abstract. *A mouse mammary tumor, adenocarcinoma BW 10232, was maintained in vitro for 14 days, separated from embryonic mammary mesenchyme by a Millipore filter. Tubules developed in the tumor; deoxyibonucleic acid synthesis declined; and a presumptive acid mucopolysaccharide matrix, not evident in the controls, appeared.*

The importance of interactions between epithelium and mesenchyme during embryogenesis has been well documented (*1*). However, there have been few investigations of the effects of inductively active embryonic tissues on the growth of tumors. These studies have demonstrated the following: cell organization in a murine ascites tumor, murine mammary tumor, and some human tumors exposed to chick notochord, neural tube, and mesonephros; emergence of cartilagenous tissue from chick and human sarcomas cultured with chick notochord; one instance of differentiation in 28 cultures of rat renal tumor incubated with rat or mouse neural tube; and generation of nephron elements in a primitive human renal tumor exposed in vitro to fetal mouse neural tissue (*2*). Our studies demonstrate that embryonic murine mammary mesenchyme produced histological evidence of differentiation in a murine breast cancer.

A syngeneic mouse mammary tumor, adenocarcinoma BW 10232 (Jackson Laboratory), carried in C57BL/6J mice, was maintained in organ culture in direct combination with, or on the opposite side of a filter to, embryonic murine mammary mesenchyme tissue. Pieces of tumor no larger than 0.3 mm were cultured for 14 days on a membrane filter (Millipore, type TH, 25 ± 1 μm thick, 0.45-μm pore diameter) in plastic organ tissue culture dishes (Falcon) (*3*). Cultures were immersed in a defined medium composed of Eagle's basal medium with Earle's salt solution (87 percent); horse serum (10 percent); penicillin (100 unit/ml), streptomycin (100 μg/ml), mycostatin (100 unit/ml) (GIBCO), and, either 3 percent or 20 percent of 9-day chick embryo extract (EE) that had been centrifuged at 800*g* to remove debris. Cultures were maintained in a 5 percent CO_2, high-humidity incubator at 37°C.

Mammary mesenchyme was obtained from embryos of CF_1 Swiss white mice which were derived from a breeding colony. On the day 12 of gestation the skin bearing the mammary gland rudiments was peeled from underlying mesenchyme so that the mammary mesenchyme could be freed from the rib anlagen. Small pieces of viable mesenchyme were placed adjacent to the tumor on the filter or held in place by clot (ratio of EE to chicken plasma, 1 : 1) across the filter from the tumor.

The tumor in vivo is characterized histologically by a stable morphology of compact cords of tumor cells with frequent mitoses (Fig. 1A). During an extended organ culture of 144 controls (71 in three percent EE and 73 in 20 percent EE) without embryonic inductive tissue, the tumor cells became randomly oriented (Fig. 1B). Only 2 of 144 control cultures presented any evidence for tubule formation, the presence of which was regarded as the minimum criterion for differentiation. Both of these controls were maintained in medium supplemented with 20 percent EE, which itself has been shown to promote DNA synthesis and support differentiation in vitro of pancreatic epithelium (*4*).

Histological evidence for differentiation was obtained after exposure of the tumor to embryonic mammary mesenchyme tissue either by direct combination or in a transfilter culture. Direct combination with mammary mesenchyme resulted in tubule formation in 8 of 24 tumors (33 percent)