Vroman (*6*) state that platelets adhere preferentially to glass coated with unmodified fibrinogen. Others (*7*) found a similar correlation; however, it is not a perfect one. For example, on some positively charged surfaces, such as surfaces coated with protamine sulfate and 2-hydroxy-3-methadcryloyloxypropyltrimethylammonium chloride polymer, fibrinogen appeared to be adsorbed out of plasma, but on a surface coated with polybrene fibrinogen was not adsorbed. On the other hand, platelets did not adhere to the surfaces that were coated with protamine sulfate (*8*).

Suggesting that simple net charge determines thrombogenicity is as disrespectful to the complexity and beauty of nature as suggesting that it is simply our body weight which keeps us alive.

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We are in complete accord with Vroman's remarks that little agreement exists among the investigators in this field regarding the relative importance of protein adsorption, platelet adhesion, and mechanical effects in thrombus formation. Vroman implies that we suggest ". . . simple net charge determines thrombogenicity. . . ." We did not make such a generalization, and we hope that our report (*1*) was not taken in that context by others. However, we are suggesting that net surface charge is a factor in the adsorption of blood proteins, and therefore may well be a contributing factor in thrombogenicity.

Baier *et al.* (*2*) have made it clear that formation of an adsorbed protein film precedes thrombus formation at foreign surfaces. It is not unrealistic to believe that this initial protein film may serve to propagate some thrombogenic property from the surface to the blood. Sawyer (*3*) has observed that various metals tend to thrombose in an apparent relationship to their position in the electromotive series, the noble metals being the most thrombogenic. Epstein and Dalle-Molle (*4*) have examined the surface charge characteristics of low-temperature, isotropic, pyrolytic carbon, a material which, when highly polished and clean, displays excellent thromboresistance. Their results indicate that this material has a high point of zero charge [approximately +100 mv relative to a saturated calomel electrode (SCE) in plasma and +300 mv relative to an SCE in 0.89 percent (by weight) NaCl] and that it exhibits a very slightly negative surface charge at its rest potential in both plasma and saline (*4*).

It was our intention in our report (*1*) to show adsorption at the solid-liquid interface *directly* using infrared internal reflection spectroscopy, while carefully controlling and reporting the actual surface *charge*, as well as the applied potential, of the metal. The fact that we used a crude fibrinogen preparation unfortunately draws attention away from our goal. The use of plasma would have been more in accord with past practice; however, as chemists, we felt more comfortable using a solution with a bit simpler composition. We believe that the relative merits of ellipsometry versus computer-assisted infrared internal reflection spectrometry are beside the point. In subsequent experiments, using the same cell (and 60 percent clottable fibrinogen) but with the addition of a minicomputer to average spectra and obtain difference spectra, we have determined that enhanced protein adsorption occurs on germanium at potentials much lower than the −200 mv observed in (*1*). At the reported point of zero charge of germanium, −900 mv, no enhanced adsorption is observed for as long as 5½ hours, although strongly enhanced adsorption is observed rapidly at −350 mv (relative to an SCE).

Recognizing the limits of our technique, we readily acknowledge that ellipsometry is capable of detecting very thin (about 5 Å) films. Infrared internal reflection spectrometry offers an advantage over ellipsometry in that it provides qualitative information on the composition of the adsorbed film. When used in conjunction with a dedicated minicomputer, it can provide quantitative measurements on films in the 25- to 1000-Å range. In either case, identification of the adsorbed proteins must depend on the use of specific antisera, as Vroman mentions.

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"γ-Glutamyl Cycle"

In setting forth a proposal of a γ-glutamyl cycle in amino acid transport (*1*), Meister and co-workers did not mention a much earlier proposal of a similar mechanism (*2*). That earlier proposal was limited to tissues concerned with transmural transport because many differentiated tissues quite active in amino acid transport were known to be devoid of the activity (*3*); the system is also found in certain fetal and dedifferentiated tissues (*4*). The proposal was abandoned in the course of later work because the specificity of the system was not found to correspond to the specificity of amino acid transport by renal tissue. Although isozymes (at least five) are present in renal tissue, they do not vary significantly in specificity and many readily transported amino acids and analogs are poor substrates (*5*). We have found the effects of Na^+ to be highly variable and dependent upon the state of the preparation; the purified enzymes are insensitive to Na^+ when tested with

glutathione as the donor and glycylglycine as the acceptor of the γ-glutamyl grouping (*5*).

There appears to be no particular reason to single out the γ-glutamyl transfer system. Tissues engaged in transmural transport have membrane-bound peptidases that could be fitted quite readily into theoretical transport schemes for amino acids or peptides.

Inasmuch as peptides are much better acceptors of the γ-glutamyl grouping than the constituent amino acids (*6*), it could be suggested that γ-glutamyl transfer is involved at some stage in the processing of peptides by transmural transport cells. It is to be emphasized, however, that there is no evidence for the formation of intermediate compounds in the transport of amino acids or peptides. Several quite plausible mechanisms for amino acid transport not involving the formation of intermediate compounds have been formulated (*7*) and similar mechanisms appear to apply to peptide transport in the intestine (*8*).

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The studies reviewed in (*1*) indicate the existence of the γ-glutamyl cycle in a number of mammalian tissues and thus seem to have elucidated a significant pathway of glutathione metabolism. The finding that 5-[^{14}C]oxoproline is actively metabolized, the discovery of 5-oxoprolinase (*2*), and the observation that kidney contains high levels of the enzymes required for glutathione synthesis stimulated proposal of the cycle. In an earlier report from our laboratory (*3*) we considered several possible functions for γ-glutamyltranspeptidase and γ-glutamylcyclotransferase (that is, protein synthesis, amino acid transport, collagen formation); while such ideas had been expressed previously, the functions of these enzymes nevertheless remained in doubt. The report in which the γ-glutamyl cycle was proposed cited the experiments on 5-oxoproline metabolism, and stated (*4*):

> Although we and others have speculated that γ-glutamyltranspeptidase might possibly be involved in amino acid transport, this now seems much more attractive because the action of this enzyme can now be integrated with the reactions catalyzed by γ-glutamylcysteine synthetase, glutathione synthetase, and γ-glutamylcyclotransferase.

We have been aware of earlier suggestions about a possible function of γ-glutamyltranspeptidase in amino acid transport, including those made by Binkley (*5*) and Hird (*6*), and have cited these (*7*). The idea that γ-glutamyltranspeptidase might be involved in amino acid transport has evidently not been mentioned in reviews by investigators who have intensively studied amino acid transport. Heinz (*8*) recently stated:

> Almost nothing is known about the chemical nature of the mechanism of amino acid transport. The participation of a mobile carrier is strongly suggested . . . this carrier is able to bind the amino acid and to carry it across the osmotic barrier and is degraded to an inactive form when delivering its substrate on the trans side. The degraded form must be returned and at the same time reactivated, opssibly by direct coupling to a metabolic reaction.

It seems highly probable that such a mechanism would involve enzymes, quite possibly those of the γ-glutamyl cycle.

Binkley and Johnson cite a report by Binkley and Nakamura (*9*), in which it is concluded that the transpeptidase is present only in kidney. However, such a limited distribution has not been confirmed by later work and we now know that the transpeptidase is widely distributed (as is glutathione), and that the enzymes of the γ-glutamyl cycle are present in choroid plexus (*10*), intestine, ciliary body (*11*), and in many other tissues (*12*). We also now know [see, for example, (*10*)] that the amino acid specificity of the transpeptidase is much broader than was thought earlier (*13*), and the γ-glutamyl cycle could therefore account for renal reabsorption of many amino acids. Although Na^+ does not seem to exert specific effects on the enzymes of the γ-glutamyl cycle, the significance of this finding is not certain; results on the Na^+-dependence of amino acid transport are inconsistent, and definite conclusions as to the role of Na^+ in amino acid transport do not yet seem possible (*14*).

It was specifically stated (*1*) that we are not proposing that the γ-glutamyl cycle is the only mechanism for amino acid transport and that there are probably a number of other systems. The possibility that the γ-glutamyl cycle functions in peptide transport and degradation was previously considered (*1*), and continues to interest us. The statement of Binkley and Johnson that "peptides are much better acceptors of the γ-glutamyl grouping than the constituent amino acids" is debatable, however, since only certain dipeptides and tripeptides, and not others, are active (*15*). Our proposal excludes neither the interesting possibility suggested by Binkley and Johnson about membrane-bound peptidases, nor the existence of amino acid transport systems that do not involve formation of intermediate compounds or utilization of carriers. However, it may be noted that intermediates have been found in the transport of other compounds such as fatty acids and sugars; the present absence of conclusive evidence of intermediates in amino acid transport may well be remedied by further work.

The function of the γ-glutamyl cycle in vivo is further indicated by work in which animals were given both a 5-oxoprolinase inhibitor and amino acids (*16*). In addition, the enzymatic defect in the human inborn error in metabolism, 5-oxoprolinuria, has been identified as a specific alteration in the γ-glutamyl cycle (*17*). We are thus impressed with the growing evidence in support of the γ-glutamyl cycle. We continue to view its proposed function in amino acid transport as "a useful working hypothesis in the design of new experiments on the enzymology of amino acid transport (*1*)."

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Laetrile and Schistosomiasis

Perhaps one of the reasons for the impression that "Laetrile 'scientists' frequently do poor science" (*1*) is that the workers who failed to demonstrate therapeutic efficacy have tended to keep their findings to themselves—thereby greatly limiting the sample size on which the impression is based.

It was predicted (*2*), on theoretical grounds, that Laetrile would be effective in the treatment of schistosomiasis (one of the most important parasitic diseases of man), and some preliminary claims were made for the efficacy of long-term Laetrile treatment in the clinic (*3*). I know of no published contrary evidence, and in view of the current resurgence of interest in Laetrile, perhaps I should here record an unpublished attempt in 1966 to demonstrate efficacy in schistosomiasis in mice (*4*).

Albino mice infected with *Schistosoma mansoni* were treated with Laetrile at a time (day 28 of infection) just prior to the expected onset of schistosome egg deposition. Groups of ten mice were subjected to one of the following regimens: 100 mg/kg per day intravenously, 100 mg/kg per day intraperitoneally, 500 mg/kg per day intraperitoneally, 500 mg/kg per day subcutaneously, or 1000 mg/kg per day intraperitoneally. In each instance the drug was given to the mice for 10 days, the days being consecutive except for the group treated intravenously, in which the 10 injections were administered over a 14-day period. A group of five mice was given potassium antimony tartrate in the diet at a concentration of 0.2 percent. Because the experiment was adjunctive to a larger trial, more than 100 additional infected mice, from the same infection batch and similarly housed in groups of five to ten animals each, received either no treatment or treatment with drugs that had no antischistosomal action. At necropsy, 28 days after the beginning of treatment, the status of infection was assessed with respect to the intensity of egg-induced granuloma formation in the liver and the presence of live schistosomes in the mesenteric veins (*5*).

All mice treated with Laetrile, in any regimen, had infections that were indistinguishable from those of the untreated mice or of the mice treated with nonantischistosomal drugs; that is, they had uniformly intense hepatic granuloma formation, the characteristic peripheral "dead worm lesions" of the liver were absent, and live worms were present in the veins. In contrast, all of the mice treated with the antimonial drug were free of hepatic granulomata (no attempt was made to determine whether all of the worms had been killed).

Thus Laetrile, as used in this experiment, neither killed the schistosomes nor suppressed their egg production (the latter usually being a sensitive indicator of antischistosomal activity). It is possible that the use of older infections or prolonged treatment would reveal an antischistosomal effect. Thus, although the therapeutic correlation between schistosomiasis in mouse and in man is quite good on a qualitative basis, these results do not rule out the possibility that Laetrile would be of value in the treatment of schistosomiasis.

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