

Studies on the Effect of a Serum Permeability Substance  
Produced During Malaria Infection on Platelets in vitro

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**OBJECTIVE:** Previous studies in this laboratory have revealed that during the course of a malaria infection, the sera of infected gibbons (*Plasmodium jeffreyi*) and monkeys (*P. inui*) produce increased vascular permeability when injected intradermally into the skin of white rabbits (see 1967 and 1968 Annual Reports). This reaction was completely abolished by antihistaminic drugs. The factor was stable to heating at 56°C for thirty minutes. Attempts at characterization of the substance were unsuccessful (see 1969 Annual Report) in part because of its apparent lability during fractionation.

Since thrombocytopenia is associated with *P. inui* infections (1969 Annual Report), the notion that the permeability factor induced rabbit platelet lysis with accompanying histamine release was entertained. Rabbit platelet lysis can be induced in vitro by immune complexes and the phenomenon detected by measurement of the resulting histamine in the fluid phase.<sup>1</sup> The possibility that the permeability factor could also induce platelet lysis in vitro was therefore explored.

**DESCRIPTION:** The assay system has been previously described (see Annual Report 1969). Briefly, sera containing activity by rabbit skin tests and sera from normal monkeys were incubated at 37°C for 60 min. with rabbit platelets in the presence of fresh autologous rabbit plasma, Ca<sup>+</sup> and Mg<sup>+</sup>. The preparations were centrifuged and the supernatants deproteinized and extracted for histamine. Histamine was converted to a fluorescent derivative and assayed fluorometrically; fluorescence intensities were related to histamine concentrations by the use of a curve prepared from a set of histamine diphosphate standards extracted and otherwise treated identically with the unknowns.

**PROGRESS:** The results of two experiments are summarized in Table I. Histamine release is expressed in terms of the total available histamine (determined by assay of an aliquot of platelets) found in the fluid phase. Spontaneous release from mixtures without serum is indicated (cell blank). Release of histamine from platelets in the presence of serum from infected monkeys (8-16%) was not greater than from control monkeys (9-23%).

These results do not preclude the possibility of platelet lysis as a mechanism of enhanced vascular permeability in rabbit skin by malarial monkey sera. However, it is considered that further studies at this time would not likely yield additional information. This is the final report on this project.

**SUMMARY:** Serum from *P. inui* infected monkeys which enhanced vascular permeability in rabbit skin failed to induce the release of histamine from rabbit platelets in vitro.

**REFERENCE:** (1) Siraganian, R.P., Secchi, A.G., and Osler, A.G., *J. Immunol.* 101: 1130, 1968.

Table 1. Histamine release from rabbit platelets in the presence of serum from *P. inui* infected monkeys. Lesions produced in rabbit skin by the same sera are shown for comparison.

Experiment No.	Test preparation		Rabbit skin lesion diameter (mm.)	% platelet histamine released
	Serum origin (Monkey #)	Days post infection		
1 Total histamine available 1.9 $\mu$ g/ml.	MS-41	preinfection	0	9
	PK-36	preinfection	0	20
	PB-1	normal	0	23
	cell blank	—	—	18
2 Total—histamine available 3.6 $\mu$ g/ml	MS-41	prior to infection	0	10
	MS-41	29 days	14	8
	PK-36	prior to infection	0	20
	PK-36	15 days	14	8
	PK-36	29 days	10	16
	PB-1	normal	0	15
	SP-2	chronic malaria	N.D.	15
cell blank	—	—	10	

N.D. = Not done.