

7. Title: Study on the identification of the substance producing increased vascular permeability during the course of malaria infection.

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OBJECTIVE

Certain globulins and polypeptides have been shown to cause increased vascular permeability. The concentration of a number of pharmacologically active peptides were found to increase during the course of malaria infection but the actual role of these substances in the disease is not understood. However, it has been suggested that they may play an important role in the alteration of body fluid compartment physiology.

Previous studies¹ revealed that during the course of a malaria infection, the sera of infected gibbons and monkeys produced an increased vascular permeability when injected intradermally into the skin of a white rabbit. This reaction was completely abolished by antihistaminic drugs.

The present study was undertaken to identify the nature of the substance producing increased vascular permeability (PIF) in malaria infected monkeys.

DESCRIPTION

Regarding the local vascular reaction of the rabbit skin injected with infected monkey serum and the inhibition of this reaction by the injection of an antihistamine, it is most suggestive that histamine is involved directly or indirectly in this phenomenon. The experiments were performed in the following sequence:

1. The attempted isolation of PIF in infected serum in pure form for pharmacological assay on the isolated animal tissue.
2. The study of the histamine releasing properties of infected serum on incubation with isolated histamine containing cell preparations.

Serum specimens collected from P. inui infected monkeys were used in all studies. Serum collected from the same monkey before infection was used to control each experiment.

PROGRESS

A) In the first part of the study, the distribution of PIF in infected monkey sera was studied by fractionation on a G-25 Sephadex chromatographic column. Eluates were characterized by polyacrylamide gel and cellulose acetate electrophoresis. Results obtained from the skin test of these eluates were correlated with the electrophoretic patterns to locate the component responsible for the PIF activity. It was found that the activity was confined to fractions containing a beta globulin demonstrable by polyacrylamide gel electrophoresis. The isolation of this protein in purified form was accomplished by a combination of salt precipitation² and DEAE cellulose column chromatographic³ techniques.

The isolated pure beta globulin and sera from control and P. inui infected monkeys were used for rabbit skin tests. The results indicated the persistence of the PIF activity in the beta globulin isolated from infected serum specimens.

Pharmacological assay using the isolated guinea pig ileum (Trendelenburg's Method)⁴ was performed. The results revealed that the P. inui infected and clean monkey sera caused an increased peristalsis and tonus of the isolated ileum. This reaction was completely abolished by application of an antihistamine. The pure beta globulin of the infected monkey inhibited the spontaneous peristaltic movement of the ileum. Attempts are being made to confirm this pharmacologic reaction.

Serum samples and their isolated beta globulin were dialysed against normal saline solution. The rabbit skin test was performed, utilizing the dialysed samples and the dialysate. It was found that both infected serum and its beta globulin lost their PIF activity after dialysis. The activity was not recovered in the dialysate. These experiments will also be repeated.

B) Attempts to Elucidate the histamine releasing property of infected monkey serum by incubation with isolated histamine containing cells.

The level of circulating thrombocytes in rhesus monkeys was studied pre—and post—infection with P. inui at weekly intervals. The direct thrombocyte counting technique described in the Army Technical Manual "Clinical Hematology" was utilized⁵. During the primary parasitemia a progressive thrombocytopenia was associated with the increase in parasitemia (Figure 1 & 2). Similar observation have been described in other malaria infections⁶⁻⁷. This finding suggested the possibility of an immune reaction between soluble immune complexes and circulating thrombocytes resulting in thrombocytic lysis. In vitro experiments⁸⁻¹³ involving the incubation of antigen and antibody with isolated rabbit thrombocytes have demonstrated a release of histamine. The finding that infected serum PIF activity in rabbit skin tests is abolished by antihistamines supported the hypothesis that PIF consists of such antigen—antibody complexes. It is well recognized that platelets are accumulated in the rabbits' skin and a direct local vascular reaction mediated by such a released histamine reaction in the skin test is suggested.

To investigate this hypothesis, experiments are being conducted involving chemical determination of histamine in supernatants of mixtures of PIF and isolated platelets in the presence of fresh plasma and divalent cations.

The semi—micro technique described by Levy¹⁴ was utilized for histamine analysis. This procedure involves deproteinization of the samples with perchloric acid and extraction of histamine into alkalized butanol, and then to an aqueous phase. The final condensation of extracted histamine with O—phthalaldehyde results in a highly fluorescent product which is estimated in the Aminco—Bowman Spectrophotofluorometer.

A series of extractions was attempted on the known concentration of histamine in standard control serum to obtain reproducible recoveries. Different concentrations of perchloric acid solution were used for the deproteinizing of the samples. It was found that a six—percent concentration offers the best results with the protein concentrations employed.

To investigate PIF mediated platelet histamine release, an in vitro system is being set up utilizing the technique described by Gocke¹⁵. Thrombocytes obtained from intracardiac puncture of white rabbits are washed with buffer and finally suspended in calcium—magnesium buffer. Fresh plasma obtained from the same rabbit is added to the platelet suspension before an incubation with the monkey serum. The supernatant of this reaction mixture is taken for histamine extraction.

These experiments are in progress.

SUMMARY

Attempts were made to identify PIF activity in P. inui infected monkey sera. A beta globulin was isolated from these sera by a combination of salt precipitation and column chromatographic techniques. Serum samples and the isolated beta globulin lost their PIF activity on dialysis against saline solution. The activity was not recovered in the dialysate.

A pharmacological assay on the isolated guinea pig ileum showed that both pre—and post infection sera caused an increase in peristaltic movements and tonus of the ileum. The application of an antihistamine completely abolished this reaction. The isolated beta globulin of infected sera inhibited the spontaneous movement of the ileum.

FIG. 1 THROMBOCYTE LEVELS AND PARASITEMIA (MONKEY M-S 41)

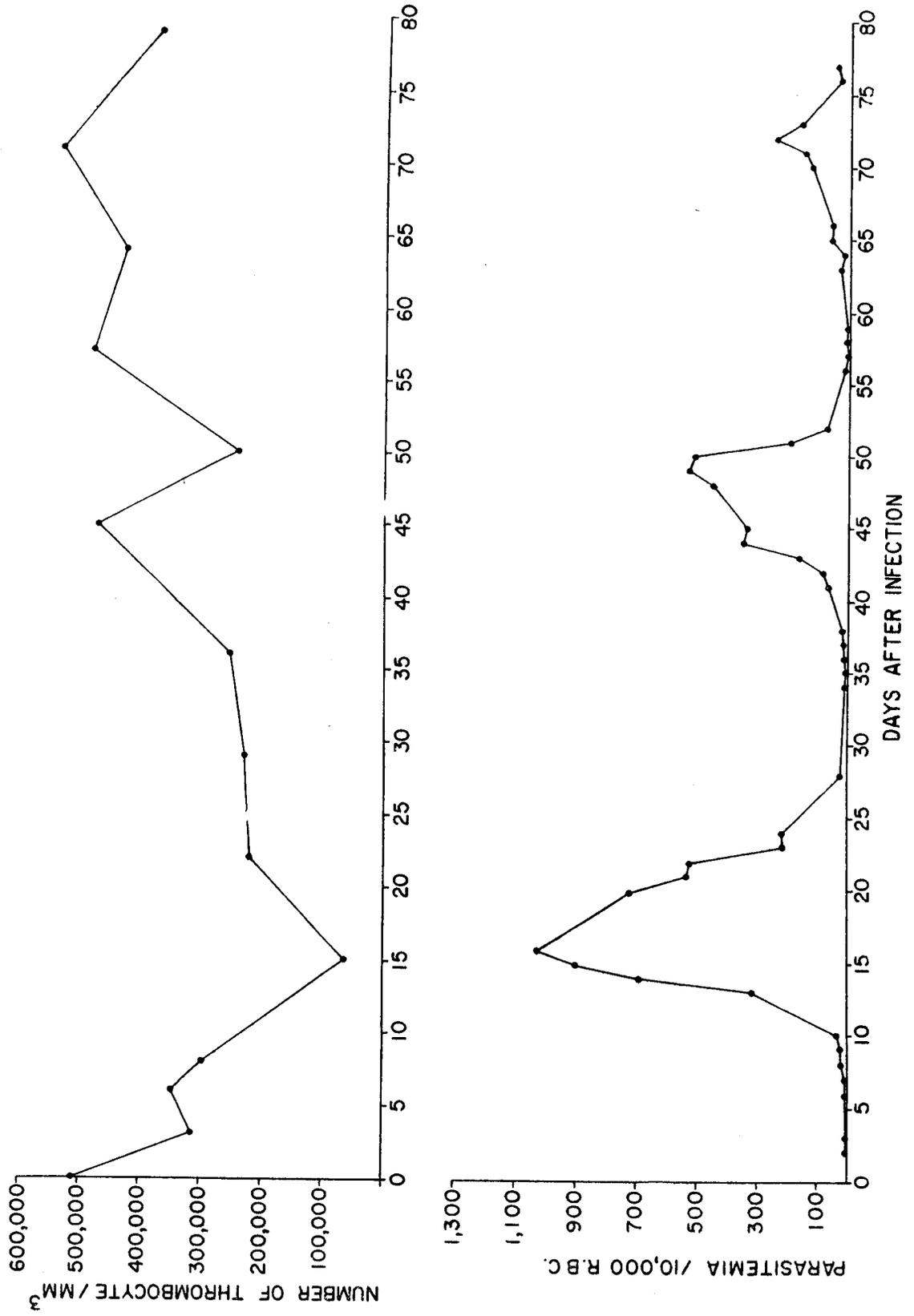
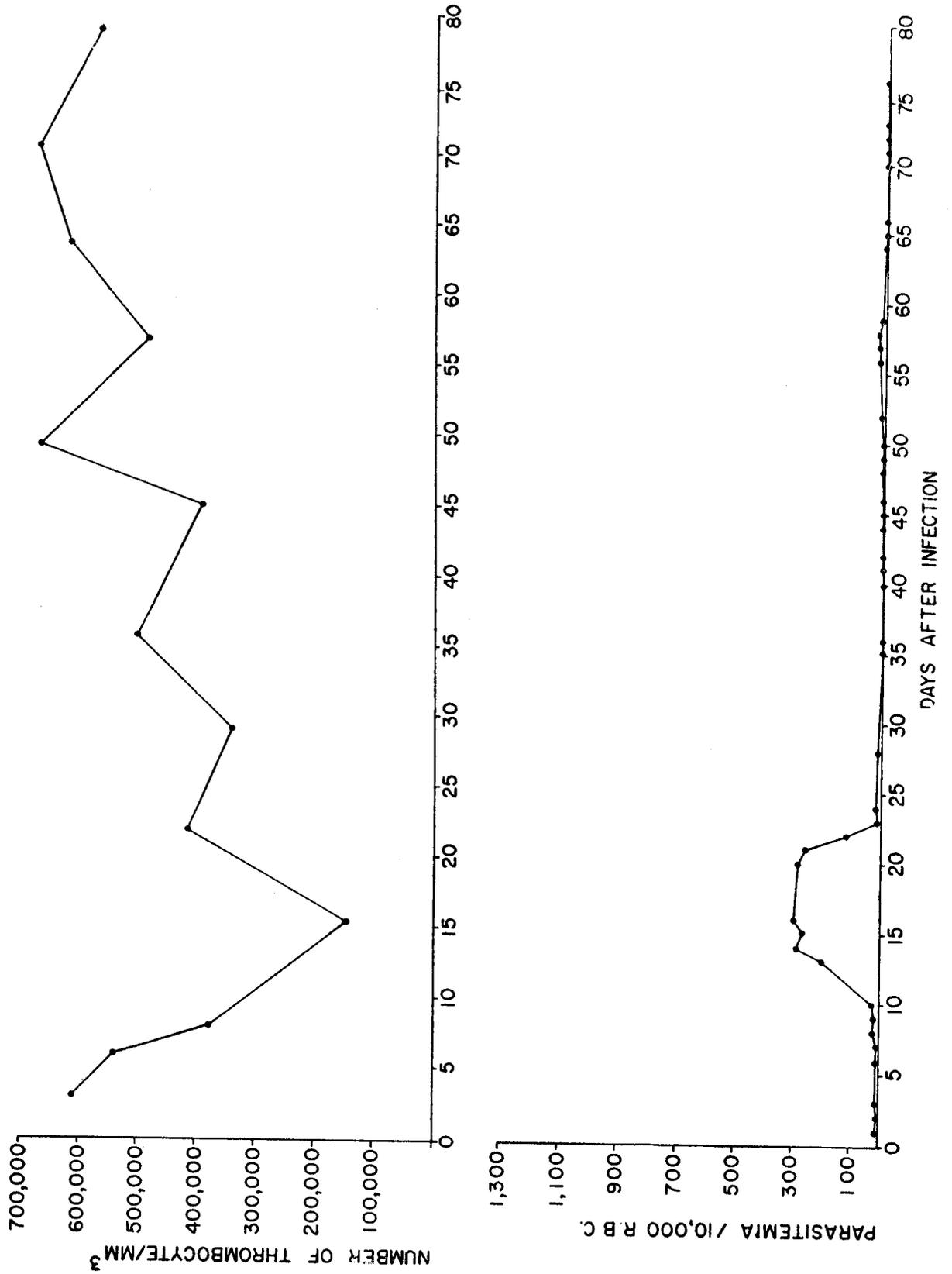


FIG. 2 THROMBOCYTE LEVELS AND PARASITEMIA (MONKEY PK-36)



A progressive thrombocytopenia associated with the increase in primary parasitemia was observed in P. inui infected monkeys.

A quantitative chemical technique for the assay of histamine is being worked out to investigate the possibility that PIF consists of antigen-antibody complexes and is capable of releasing free histamine from isolated cell suspensions.

Experiments are being carried out to elucidate the possible role of pre-existing histamine released by an immune reaction in vivo.

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