

## Serologic Studies in Human Malaria

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**OBJECTIVE:** The original objective of this study was to compare the serologic response after (a) adequate treatment of the initial attack (U.S. troops) and (b) multiple episodes of malaria (sera from endemic areas).

**DESCRIPTION:** Since antigen usable without further purification was not obtainable, lysates of washed *P. falciparum* chimpanzee erythrocytes were obtained from WRAIR and attempts were made to isolate antigenic fractions to be employed with the soluble antigen fluorescent antibody (SAFA) test (Sadun et al). Limited trials at isolation by DEAE Sephadex chromatography (Sadun et al) and more extensive efforts by G-200 Sephadex Chromatography (Gore, personal communication) were made. Fractions were monitored by absorption at 280 m $\mu$ . Serologic testing was by the SAFA test using sera from patients with proven malaria.

**PROGRESS:** In our hands, fractionation of lysate on DEAE Sephadex resulted in poor yields of protein; therefore, we turned to G-200 Sephadex. A total of four runs were made. The lysate was eluted in two major peaks as determined by absorbance at 280 m $\mu$ ; the first, eluted with the excluded volume, was colorless and opalescent; the second contained the bulk of the hemoglobin. Four eluate pools were prepared from material eluted prior to the second peak (hemoglobin): fraction I was the entire first absorbance peak; fractions II and III (FII and FIII) were prepared from the eluate between the two absorbance peaks; fraction IV (FIV) was taken from the ascending portion of the hemoglobin peak. SAFA tests employing these fractions showed no difference between normal and malaria sera in three of four chromatographic runs (#1, 3 and 4); in the second run FII and FIII gave some evidence of antigenic activity; FI yielded higher fluorescence in 5 of 8 tested malaria sera than any of the 8 normal control sera; FIII gave a stronger reaction than the controls in 4 of the 8 cases. However, results obtained on retesting five and seven days later suggested some loss of activity of the FI antigen. Further tests will be required to clarify this point.

Possible reasons for our failure to isolate active antigen consistently are (a) the lack of facilities for preparative chromatography in the cold and (b) the relatively lengthy period of storage of the crude lysate before use (more than 6 months).

Despite arrangements made with several units, only a few serum samples from U.S. troops have been obtained.

This project will be terminated on 1 July 70 due to departure of the principal investigator.

**SUMMARY:** Attempts to isolate antigen for the malaria SAFA test have been only partially successful.

**REFERENCE:** (1) Sadun, E.H. and Gore, R.W. *Exptl. Parasitol.* 23:277, 1968.