the first injection and thereafter at increasing intervals of 2 days to 3 weeks until 2 months after the last injection.

The results can be briefly summarized as follows: There was no appreciable change in the psychiatric or neurological picture, nor in the pulse rate, temperature, respiration, blood pressure, basal metabolic rate, blood cholesterol, sugar tolerance curve and kidney function. There was a marked cellular reaction in the cerebrospinal fluid. This was highest 8 hours after the injection (380 cells after 2 cc in case 1; 470 cells after 10 cc in case 2). This reaction changed within 2 days from a polynuclear one to a lymphocytic one. The cellular reaction was much stronger than could be expected from repeated lumbar puncture and it could be observed as long as 2 months after the last injection (7 cells per cmm). It was also more marked and prolonged than might be expected from equal amounts of normal saline. There was also, during the first 2 days, an increase in total protein (from 0.015 to 0.040 g %).

The long-standing cellular reaction is noteworthy because Herrmann[2] has shown that heavy water disappears from the cerebrospinal fluid spaces within 30 minutes, whereas meningeal reactions of this duration are commonly associated with the presence of slowly reabsorbed material in the cerebrospinal fluid spaces (Finlayson and Penfield[3]).

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## Precipitative Tests in Malaria.*

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We have obtained precipitative reactions in human malaria with *P. knowlesi* antigens through use of the collodion-agglutination method of Cannon and Marshall,[1, 2] and the collodion fixation procedure of Goodner.[3]

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2 Herrmann, J B., *J. Pharm. and Exp. Ther.*, 1939, **67**, 265.

3 Finlayson, A. I., and Penfield, W., *Arch. Neur. Psych.*, 1941, **46**, 250.

* This work was made possible through a coöperative program financed by the Tennessee Valley Authority and the University of Tennessee.

1 Cannon, P. R., and Marshall, C. E., *J. Immunol.*, 1940, **38**, 365.

2 *Ibid.*, 1941, **40**, 127.

3 Goodner, Kenneth, *Science*, 1941, **94**, 241.

Cannon and Marshall[1, 2] adsorbed purified antigens on collodion particles. The sensitized particles were then combined with serial dilutions of antisera and the precipitative titers thus determined. By this method precipitins have been demonstrated in sera from patients sensitive to egg protein, tuberculin and crystalline insulin. In studies of the mechanism of the Arthus phenomenon precipitative titers were found to parallel skin reactivity. Weir[4] has also used this collodion agglutination procedure to show antibody formation in animals to tubercle bacilli and tuberculin.

Goodner[2] adds collodion particles at the time of combining antigen and antibody. The adsorption of the collodion pellets by the antigen-antibody complex produces particulate agglutination which may be macroscopically estimated. Antiviral and antimalaria antibodies have been demonstrated by this method.

Our previous attempts to demonstrate a precipitative reaction by overlaying sera from malarious patients with antigens prepared from parasitized bloods of human individuals and monkeys were unsuccessful. For these tests we used saline and watery extracts of malaria parasites and blood clots, as well as papain digests and sodium hydroxide extracts of parasites. We could not show significant differences between malarious and non-malarious sera.

A saline extract of *P. knowlesi* parasites obtained from heavily infected monkeys has proved a very satisfactory antigen for use in complement fixation tests in human malaria. Eighty-two percent of 125 patients with malaria parasites in the peripheral blood gave positive reactions although approximately one-third of this group had received some anti-malaria drug. This antigen as well as the alkaline extract of parasites described below has proved successful in the two procedures employing collodion particles.

*The Use of Cannon and Marshall's Technic.* In the collodion agglutination tests we followed exactly the technic of Cannon and Marshall† in all essential details. The antigen used for the tests reported in Table I was prepared by extracting 0.1 g of dried *P. knowlesi* parasites with 10 cc of N/20-N/10 NaOH for an hour. After neutralization it was centrifuged at high speed and the supernatant used to coat the collodion particles. It is recognized that this is a crude preparation but attempts to purify it by dialysis, or treatment with precipitating reagents have been unsuccessful. Difficulties with adsorption of antigen by particles which we encountered at

[4] Weir, J. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 47.

† Dr. Cannon kindly supplied us with crystalline egg albumen and antiserum for our initial work.

times are attributed to the unpredictable variation of different lots of antigen.

The sera were used in dilutions beginning with 1:4. A control series employing the same serum dilutions and uncoated collodion particles was run simultaneously whenever the quantity of serum permitted. Table I presents the results of precipitative tests for malaria on 97 patients.

In all cases the agglutinated particles formed small clumps but the tests were easily read. We used artificial light for readings.

As shown in Table I, 29 (87.8%) of the 33 patients with malaria parasites in the peripheral blood gave a titer of 1:8, while 26 (78.7%) gave a titer of 1:16. Six (11.7%) of the 51 patients of groups II and IV who were regarded as non-malarious, gave a titer of 1:8; 2 (0.39%) a titer of 1:16. These data have been analyzed statistically and the differences between the two groups are significant.

*The Use of Goodner's Technic.* Goodner's preliminary report

TABLE I.
Results of Precipitative Tests for Malaria on 97 Patients.

| Clinical group | No. of patients | No. of sera giving titer indicated: Below 1:8 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | 1:512 |
|---|---|---|---|---|---|---|---|---|---|
| I<br>Malaria positive blood film | 33 | 2 | 5 | 6 | 9 | 3 | 5 | 2 | 1 |
| II<br>Malaria excluded by clinical diagnosis and negative blood film | 20 | 13 | 4 | 2 | | 1 | | | |
| III<br>Malaria not excluded by clinical diagnosis but negative blood film | 10 | | | †* 3 | ††* 3 | * 1 | | ** 3 | |
| IV<br>Non-malarial febrile group. Blood films not available | 31 | 29 | 2 | | | | | | |
| V<br>Febrile conditions of undetermined origin. Blood films not available | 3 | 1 | | | 2 | | | | |

† Positive complement fixation for malaria.
* Clinically malaria. Responded to treatment.

includes a table showing results of collodion fixation in virus-anti-virus systems. He combines 0.5 cc of diluted antigen, 0.1 cc of collodion particles, 0.1 cc of patient's serum and 0.3 cc saline. We have found that those quantities of reagents may be used in the precipitative test for malaria. The saline extract of *P. knowlesi* parasites has proved our most satisfactory antigen. This is prepared by grinding 0.1 g of dried parasites in saline followed by freezing and thawing for 4-6 times with dry ice and alcohol. After standing over night in the icebox the preparation is centrifuged at high speed and the supernatant used as antigen. It is not so highly colored as the alkaline extract.

We have carried out titrations of antigen and antibody in an attempt to determine the optimal ratio. Serum undiluted and diluted 1:4 in combination with antigen diluted 1:10 to 1:40 have given us satisfactory readings after an incubation period of one hour at room temperature and over night in the icebox. The collodion particles are added at the time of combining antigen and antibody. We have confirmed Goodner's finding that the collodion particles must be added at the time of combining these reagents. Negative results are obtained if the particles are added one hour later.

A control series with antigen omitted is always included. All tubes are centrifuged at 500 rpm for 5 minutes just before making the readings. Positive tests are clear-cut and are recorded at 4+ to negative.

At the present date we have records on 30 patients. Nineteen patients had malaria with positive blood films. Seventeen of these sera gave positive reactions. The 2 patients who gave negative reactions had received multiple doses of quinine. Eleven individuals gave negative blood films and malaria was not a clinical diagnosis. These individuals gave negative reactions.

*Summary.* Precipitative reactions in human malaria may be demonstrated (1) by the use of *P. knowlesi* antigen-coated collodion particles, or (2) by addition of collodion particles at the time of combining antigen and antibody. Macroscopic agglutination is obtained by both methods.