We could not find any correlation between the results of the tests and the number of attacks of malaria that the patient has had. Two soldiers came back with a subsequent attack while the study was going on. One had had a positive series of tests on the first admission and a negative series on the second. Another had negative tests on both occasions. There was also no correlation between the duration of the malaria, dated from the first attack, or between the origin of the malaria and the result of the cephalin-cholesterol flocculation test.

As stated by Mirsky[1], there was no correlation between the result of the Kahn test and the cephalin-cholesterol flocculation test, regardless of time of reading of the test.

These results do not verify the statement that all patients with malaria show impairment of liver function as measured by the cephalin-cholesterol flocculation test. The 24-hour technic shows a lower percentage of positive results than the 48-hour technic. The percentage of positive results becomes higher if repeat tests are done, but never higher than 75%. The cephalin-cholesterol flocculation test is not an absolute differential diagnostic point between malaria and sand fly fever.

15120

Further Observations on Red Cell Agglutinating Agent Present in Lungs of Virus-Infected Mice.

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In a previous communication[1] we have reported the presence of a heat-stable hemagglutinating agent in the lungs of mice infected with a pneumonitis virus. This agent agglutinates murine erythrocytes and the agglutinin is inhibited by specific antiserum. The agglutinating property becomes manifest only after treatment of infected lung extract by heat.

The work reported in this paper, and the previous one, has been carried out with the "ES" strain of pneumonitis virus, a strain nearly identical with the pneumonia virus of mice, described by Horsfall and Hahn.[2,3] The hemagglutinating agent is regularly present in the lungs of infected mice. It has been demonstrated in mice killed 48 hours after intranasal inoculation, is present in its highest titer 5-6 days after infection and is usually absent in mice surviving 9-10 days or longer.

Preparation of the agglutinating lung extract has been simplified. Each pair of infected lungs is ground with sand and 2 cc of normal salt solution. The suspension is heated for 10 minutes in a water bath at 75-80°C, centrifuged, and the supernatant fluid removed for study. Various tests have been performed in order to determine the nature of the agglutinating agent and the conditions under which the agglutinating property is masked or unmasked.

The results in Table I indicate that the max-

TABLE I.
Range of Temperature Causing Maximum Activity of Agglutinating Property of Lung Extract.

Degree of heat °C	Time, min.	Agglutination (titer)
Untreated	—	0
56	30	0
60	15	±
65	5	±
70	5	1-64
75	5	1-64
80	5	1-32
85	5	1-16
90	5	±
95	5	trace
100	5	0

1 Mills, K. C., and Dochez, A. R., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 140.

2 Horsfall, F. L., and Hahn, R. G., PROC. SOC. EXP. BIOL. AND MED., 1939, **40**, 684.

3 Horsfall, F. L., and Hahn, R. G., *J. Exp. Med.*, 1940, **71**, 391.

imum agglutinating power of the extract develops after heating for 5 minutes at temperature ranges from 70°-80°C and that the agent is remarkably resistant to heat.

The red cells of a number of animal species have been examined for their agglutinability. Those yielding positive results are the red cells of the mouse and the hamster. Those yielding negative results are human group O, fowl, guinea pig, rat, cotton rat, sheep, cat, dog, and ferret. Rabbit cells show slight but irregular agglutination.

The agglutinating agent, in the state in which it exists in fresh lung suspensions, retains its activity for at least 7 days at room and at ice-box temperatures. Heated extracts prepared from such preserved suspensions exhibit undiminished agglutinating capacity.

The inability of fresh unheated lung extracts to cause agglutination of mouse red cells seems to be due to masking of its activity by substances derived from blood or lung tissue in the process of maceration by grinding. In order to test this hypothesis substances which might be present in fresh mouse lung were added to a heated agglutinating extract and their capacity to affect the agglutination phenomenon was observed. The addition of normal mouse serum to heated agglutinating extract resulted in no diminution of agglutinating capacity. On the other hand, when washed murine red cells were added to such an extract, allowed to stand for 1 hour at room temperature, and the mixture then centrifugalized and the supernatant fluid tested for agglutinating capacity, this power was either completely lost or markedly diminished. High concentrations of red cells 33-40% effected complete loss, 5-10% very marked diminution and 1.25-2.5% moderate to slight diminution of the agglutinating power of the extract. Removal of the cells by centrifugation and retreating of the supernatant fluid at 80°C did not result in restoration of the agglutinating capacity. Treatment of fresh lung suspensions in the same manner resulted, upon removal of the red cells, in retention of undiminished infectivity and the development of a high agglutinating titer by the extract after heating. In short, the addition of a large volume of suitable red cells to heated agglutinating extract completely absorbs out the agglutinating agent, whereas a similar procedure applied to untreated lung suspensions leaves, upon subsequent heating, the agglutinating capacity unchanged.

The agglutinating agent present in heated lung extract can also be completely absorbed and removed by means of laked murine red cells, mouse stromata, and by ground up fresh normal mouse lung.

Other methods of preparing an active agglutinating extract such as: thorough perfusion of the lung before removal, exposure of the suspended lung tissue to 15% sodium chloride, 5% formalin, 1% calcium chloride, half saturation with ammonium sulfate, and 50% alcohol did not result in the development of agglutinating power by the lung tissue suspension.

Horsfall and Hahn have reported that mice can be actively immunized against the pneumonia virus of mice by intraperitoneal injections of living virus. It seemed of interest therefore to determine if the heated virus-containing extract discussed above has the capacity to induce active immunity in mice. Mice were given at 4-day intervals three injections of 0.5 cc of heated extract. Seven days after the last injection the mice were challenged by intranasal inoculation of a 50% mortality dose of living virus. All mice previously injected with extract were solidly immune, whereas all controls developed characteristic lung infections. Furthermore, the serum of mice immunized against the extract inhibited to high titer agglutination of red cells by active extract. In our stock, normal mouse serum possesses no such inhibitory power.

Rabbits immunized with living E.S. virus or the standard pneumonia virus of mice rapidly develop antibodies capable of neutralizing the agglutination reaction. Serum of a rabbit immunized with heated lung extract neutralized the agglutinating power of the extract at high titer and the infectivity of living virus at a somewhat lower level.

Horsfall[4] has noted the infectivity of pneumonia virus of mice for hamsters. Inoculation of mouse lung suspensions containing

[4] Horsfall, F. L., Personal communication.

the E.S. virus into the Syrian hamster frequently causes large areas of pulmonary consolidation and occasionally death of the animal. Serial passage, with irregular exceptions, could not be successfully achieved. Agglutination of red cells exuding from consolidated hamster lungs was regularly observed and heated extracts of these lungs agglutinated both mouse and hamster red cells in high titer. Normal serum from these hamsters had a low capacity for inhibiting red cell agglutination, whereas the serum of animals recovering from infection acquires this power readily. In contrast, the serum of recovered mice acquires the capacity for agglutinin-inhibition more slowly—about 9 days after infection. It is possible that the early development of immunity by the hamster interferes with the spread of infection in this animal.

Dr. David E. Green and Miss Violet Nocito have undertaken the purification of the agglutinating agent.

The agent was readily purified by successive precipitations with ammonium sulfate. At 20-35% saturation of ammonium sulfate inactive proteins were removed whereas the agglutinating agent precipitated out in the saturation range 35-50%. Solutions thus purified were colorless and clear, and were approximately 15 times as active as the original heated extract per unit of protein. The immunizing capacity of the agglutinating agent tested at this purity level was found to be unimpaired. The studies on the purification of the agglutinating agent are being continued and will be reported elsewhere.

Summary. The red cell agglutinating agent present in the lungs of mice infected with pneumonitis virus has been subjected to further study. Maximum agglutination develops after heating the fresh lung extract at 75°C for 5 minutes. The agent is absorbed from active extracts by preparations of mouse red cells and of fresh normal mouse lungs. Mice injected with the heated agent develop active immunity against the living virus and their sera have the capacity of inhibiting red cell agglutination. The sera of rabbits immunized against living pneumonitis virus or against active heated mouse lung extract inhibit red cell agglutination. Hamsters appear to be susceptible to infection with the pneumonitis virus but attempts at continued serial passage were unsuccessful. The agglutinating agent has been purified by precipitation with ammonium sulfate. The purified agent retains its agglutinating and immunizing capacity.

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Artificial Induction of Subcutaneous Nodules in Rheumatic Fever.*

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During the course of studies on various aspects of rheumatic fever a working hypothesis was developed which postulated that this syndrome is one of the manifestations of the uninhibited action of proteinases on mesenchymal tissues. It was proposed that these proteolytic enzymes are either activated *in vivo* by streptococcal fibrinolysins or result from the release of intracellular proteinases consequent to the cellular damage produced by infection, trauma, or "anaphylactoid" reactions. Further, it was assumed that the individual who is susceptible to the development or recurrence of rheumatic fever is one who cannot inhibit adequately the proteinases thus activated. The present report is concerned with the application of this hypothesis to a study of the induction of subcutaneous nodules in susceptible subjects.

Although Drewitt[1] postulated in 1883 that trauma was involved in the production of subcutaneous nodules in rheumatic fever, it

[1] Drewitt, F. D., *Brit. M. J.*, 1883, **1**, 622.