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.

was not until 1937 that evidence for this concept was reported. At that time Massell, Mote and Jones[2] demonstrated that the injection of 2 to 3 cc of whole blood subcutaneously over the anesthetized olecranon process, with subsequent frictional pressure, resulted in the appearance of a nodule in the injected region in 90% of those patients with clinically active rheumatic fever, in 50% of those with only laboratory evidence of active rheumatic fever and in only 14% of those rheumatic fever patients who had no evidence of active disease. Accordingly, these investigators suggested that tissue injury, if sufficient, may be of primary importance in the spontaneous production of a nodule in the patient with acute rheumatic fever, though some specific effect of blood itself could not be ruled out.

It is apparent that the destruction of cells by trauma will result in the release of their intracellular enzymes, which, if not inhibited by proteinase-inhibitors, may in turn produce the secondary changes responsible for the appearance of the nodule. Accordingly, we studied the influence of the subcutaneous injection of trypsin on the induction of a nodule. It must be emphasized that although trypsinases are known to be present in cells, it is quite possible that some other proteinase may prove to be involved.

Four groups of male subjects of from 19 to 40 years of age were investigated. The first group consisted of men who were recovering from a variety of clinical disturbances but who gave no personal or family history suggestive of rheumatic fever and did not present any evidence of a recent streptococcal infection. They are designated as "non-streptococcal controls." The second group was comprised of subjects who had had B hemolytic streptococcal infections (as proven by positive cultures) 2 to 3 months before the present study. None of these patients developed any indications of rheumatic fever, and are designated as "streptococcal controls." The third group was made up of subjects who gave a definite personal or family history of rheumatic fever, but were hospitalized, at the time of the study, for some other reason. This group is designated as "rheumatic fever history." The fourth group consisted of men who had developed signs and symptoms of rheumatic fever but were convalescing at the time when the present study was performed.

The majority of subjects were injected subcutaneously over the ulna with 1 cc of 1% solution of crystalline trypsin magnesium sulfate compound in either saline, M/400 HCl, or water, while a number of patients were injected with 1 cc of a 5% solution of crude trypsin (Wilson). All solutions were filtered (Seitz) before use. No difference was noted between the effects of the crystalline and crude preparations except that a concentration of less than 5% of crude trypsin was ineffective.

Edema usually developed at the site of the injection in from 2 to 3 hours and in some instances (usually the rheumatic fever subjects) persisted for as long as 48 hours. Those subjects who developed nodules usually developed the lesion at the site of injection in from 4 to 7 days. Thus some subjects showed no evidence of a real nodule until the 7th day after the injection, while others developed one by the 4th day. The nodule had a definite contour and was readily distinguished from the indefinite induration which occurred at the site of injection in about one-third of all subjects. In the proper light, the nodule was easily detected.

The size of the induced nodules varied a great deal but this feature could not be related to activity nor to any clinical or laboratory observation. The duration of the nodules also varied markedly: from a few days to many weeks.

The subcutaneous injection of trypsin did not induce any change in either blood morphology or erythrocyte sedimentation rate. A number of rheumatic fever patients volunteered the information that they experienced fleeting pain in various joints during the first 48 hours after the injection, but the significance of this cannot be evaluated at the present time.

The preliminary data are summarized in Table I and suggest that subjects who are convalescing from rheumatic fever or those who have had a personal or family history of this syndrome respond to the subcutaneous injec-

[2] Massell, B. F., Mote, J. R., and Jones, I. D., *J. Clin. Invest.*, 1937, **16**, 126.

TABLE I.
Induction of Subcutaneous Nodules with Trypsin.

Group*	Total No.	Positive	
		No.	%
Non-streptococcal controls	75	10	13.3
Streptococcal controls	40	1	2.5
Rheumatic fever history	22	17	77.3
Rheumatic fever convalescents	145	102	70.3

* See text.

tion of trypsin with the production of a nodule. The fact that 13% of the "non-streptococcal controls" developed nodules while 30% of the "rheumatic fever" subjects did not do so may reflect an inadequate history or a faulty diagnosis.

It is of interest to note that of the 40 patients who had had a previous streptococcal infection, without developing rheumatic fever, only one developed a nodule at the site of the trypsin injection. This observation is subject to a variety of interpretations. Thus it may be inferred that since this group of patients did not develop rheumatic fever, in consequence of their streptococcal disease, they represent subjects who are not "susceptible" to the disease. On the other hand, since a sulfonamide resistant strain of (Type 17) B hemolytic streptococci was isolated from the majority of these patients, it is possible that they were not "sensitized" to some streptococcal factor present in other strains of the organism. However the fact that infection with this strain of streptococci has been followed by rheumatic fever in some cases that have been reported suggests that a lack of streptococcal sensitization is not responsible for the negative results.

The specificity of the response to trypsin must await further study. Thus a small group of patients with rheumatoid arthritis behaved like the rheumatic fever group. This may indicate that both diseases are different manifestations of one disorder, as is suspected by many investigators, or may reflect the nonspecificity of the response to trypsin. However, it is of importance to note that since the appearance of a nodule is not related to the presence of active disease, it is improbable that we are dealing with some general sensitization but that the response may be related to whatever phenomenon is responsible for the susceptibility to rheumatic fever.

A few experiments with trypsin which was previously inactivated with "trypsin-inhibitor" suggest that the response noted above is due specifically to the trypsin. Further, our data indicate that the nodules produced by Massell, Note, and Jones are not due to the blood which they injected but probably to the tissue damage that was thereby induced. However, more extensive studies must be made before any conclusions can be reached as to the validity of the hypothesis outlined at the outset, and as to whether the induction of subcutaneous nodules has any prognostic or diagnostic significance.

Summary. The subcutaneous injection of a solution of trypsin induced "nodules" at the site of injection in 70% of patients convalescing from rheumatic fever while only 13% of apparently normal subjects responded in a similar manner.