1:1, induced fatal scrub typhus infections in 4 of 6 inoculated mice and produced immunity to challenge with *R. tsutsugamushi* in the 2 survivors. It is noted that if antibody directed against *R. tsutsugamushi* was present in the blood of Monkey 13 on day 10, this antibody was present in an amount insufficient to suppress rickettsemia in this animal. However, in spite of the occurrence of rickettsemia on day 10, no lesion developed at the site of the second injection where one would have been expected to occur if immunity factors were not present and active in the skin.

Observations on febrile, serologic and challenge responses. With one single exception, all monkeys responded to the inoculum with development of Weil-Felix and complement-fixing antibodies. Failure of one animal to respond serologically (Monkey 11 in Table I) suggests that this monkey might have escaped systemic infection; this belief is substantiated by the finding that this animal experienced only a single day of fever (day 4) after rickettsial exposure, whereas all other monkeys underwent 2 or more days of fever. All 9 animals resisted a challenge inoculum of 10,000 mouse ID_{50} of strain Karp administered 77 days after administration of the first inoculum. All remained afebrile and free of specific dermal lesions. It is noted that even though Monkey 11 might have escaped infection of sufficient intensity to elicit formation of detectable antibodies following initial exposure to *R. tsutsugamushi,* the 3 injections of rickettsiae administered on days 0, 3 and 6 were enough to render this monkey immune to the relatively massive challenge inoculum administered 2½ months later.

Discussion and summary. These findings provide evidence that immunity factors were active in the skin in the immunologic defense of rhesus monkeys experimentally infected by the intradermal route with scrub typhus rickettsiae, 6 days after exposure to the rickettsiae and several days before circulating antibodies had reached levels high enough to rid the blood of organisms. In 3 groups of monkeys, development of specific dermal lesions was completely suppressed at sites of the second, third, and fourth injections of scrub typhus rickettsiae when an interval of 6 days separated the first from subsequent injections, while in a single group of monkeys (Group 1) given 3 injections at a 3-day interval, lesions occurred at 2 injection sites. Although the role of circulating antibodies in the state of resistance to superinfection in the skin is unknown, the fact that presence of the immune state in the skin of a single monkey was established at the time this animal was circulating rickettsiae in the blood favors the suggestion that other immunity factors besides circulating antibody were at work in the dermal resistance of monkeys inoculated by the technique employed.

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Effects of Urea and Some Organic Solvents on Myosin A.* (30287)

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A relatively large number of compounds is known to cause activation or inhibition of myosin A-ATPase depending upon their respective concentrations in the incubation mixture. Some of these modifiers, such as ethylene diamine tetraacetic acid (EDTA), p-

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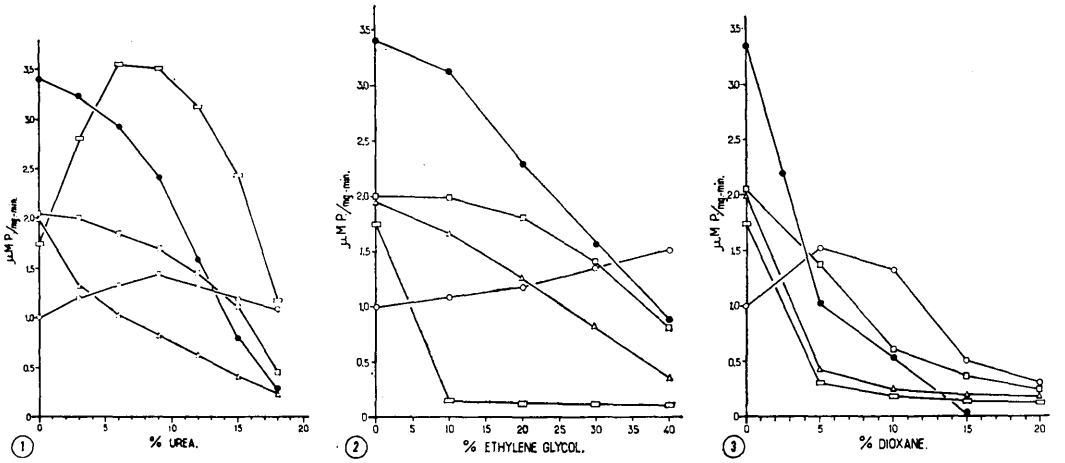

FIG. 1. Effect of urea on Ca^{++}-ATPase and Ca^{++}-ITPase activity of myosin A in presence and absence of various modifiers. Each tube contained 0.15 mg myosin A, 5 μM ATP and 50 μM tris buffer (pH 7.5) in a 1.0 ml total volume to which the following additions were made: ○ 5 μM Ca^{++}, □ 5 μM Ca^{++} + 5 μM DNP, △ 5 μM Ca^{++} + 0.05 μM PCMB, ▭ 1 μM EDTA, ● 5 μM ITP (No ATP!).

FIG. 2. Effect of ethylene glycol on Ca^{++}-ATPase and Ca^{++}-ITPase activity of myosin A in presence and absence of various modifiers. Content of tubes as well as additions and symbols used are the same as those in Fig. 1.

FIG. 3. Effect of dioxane on Ca^{++}-ATPase and Ca^{++}-ITPase activity of myosin A in presence and absence of various modifiers. Content of tubes as well as additions and symbols used are the same as those in Fig. 1.

mercuribenzoate (PCMB), 2,4-dinitrophenol (DNP), are believed to interact with certain "specific groups" in or close to the active center of the enzyme. The mechanism of action of some other compounds such as urea, ethylene glycol and dioxane was explained mainly on the basis of their respective effects on the secondary or tertiary structure of the protein molecule. It was, therefore, thought that a study of the effects of some "specific" modifiers in the presence and absence of the specified solvents may throw more light on the importance of hydrogen and hydrophobic bonds in the enzymatic reactions of the myosin A.

Myosin A was prepared by standard methods(1). All ATP and ITP splitting experiments were carried out at 37°C in a 0.05 M tris buffer. The tecnique for ATPase determination has been published(2).

It may be seen from Fig. 1 that urea concentrations between 6 and 12% doubled the EDTA-activated ATPase activity of myosin A while that of Ca^{++}-ATPase was accelerated to a much smaller extent. Six per cent urea completely abolished the PCMB activation but diminished the DNP effect by only 20%. These results showed clearly that even small amounts of urea caused alterations of the active center of myosin A, so as to produce enzymatic effects which were decidedly different in the presence of various modifiers. The fact that urea enhanced only the EDTA-activated-ATPase would suggest that hydrogen bonding, as it existed on the enzyme, did not provide the best fit for ATP to be split in the presence of EDTA.

If ethylene glycol was used instead of urea different results were obtained. Fig. 2 shows that the EDTA-activated ATPase of myosin A was completely inhibited by 10% ethylene glycol while the Ca^{++}-ATPase remained unchanged. The DNP and PCMB moderated Ca^{++}-ATPase were both depreseed by increasing concentrations of ethylene glycol.

The effects of dioxane were then studied. Dioxane, 5% concentration, accelerated Ca^{++}-ATPase but strongly inhibited its activity in the presence of 0.5 mM PCMB and diminished the activating effect of 5.0 mM DNP. The EDTA activated-ATPase was also inhibited (Fig. 3).

Based upon kinetic analysis, it has been widely believed that modifiers like PCMB,

DNP and EDTA, transformed the active center of myosin A ATPase to one similar to that which functioned with ITP as the substrate(3,4,5). With the solvents employed, Ca^{++}-ITPase indeed behaved very similarly to the PCMB modified Ca^{++}-ATPase. However, in urea, dioxane and to some extent in ethylene glycol, its behavior was quantitatively different from the DNA moderated Ca^{++}-ATPase and in urea and ethylene glycol, it was entirely different from the EDTA-activated ATPase (Fig. 1 to 3).

The previously described experiments demonstrated that compounds capable of interacting with hydrogen and hydrophobic bonds may alter the enzymatic function of the active center of myosin A in the concentration range where their effects on the secondary and/or tertiary structure of the protein were very small(6,7,8,9). The quantitatively different sensitivities of the DNP and PCMB modified enzyme site toward the various solvents investigated could serve as further proof that these modifiers which differ chemically act through different groups of the protein (4,10). These results also suggest that minor structral changes, even at some distance from the locus where ATP is being split, may change the "flexibility of the active site." In the case of this enzyme, the space occupied by the substrate on the protein molecule is probably much smaller than the "conceptual" active site.

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Effect of Ascorbic Acid on Tissue Deposition of Chloroquine in the Guinea Pig. (30288)

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The problem of the so-called chloroquine* (CQ) retinopathy, originally pointed out by Hobbs, Sorsby, and Freedman(1), has become increasingly important in recent years as a result of the widespread use of this drug at high daily dose levels for alleviation of such conditions as rheumatoid arthritis(2) and systemic lupus erythematosus(3). The incidence of retinopathy is not especially high (4,5), but it is a very serious complication for those so afflicted. The condition appears to be reversible if detected in time, and medication is discontinued(6,7); nevertheless it is obvious that effective methods for accelerating the elimination of CQ would be desirable. The use of such agents as ammonium chloride has been suggested(5); this compound has long been known to produce such an effect(8). It is also known that CQ has a marked affinity for the eyes of pigmented rats, undoubtedly due to their relatively high content of melanin(5,9,10). Recently Perez *et al*(11) demonstrated that the binding of CQ to melanin is decreased by ascorbic acid. This suggested 2 possibilities: 1) retinopathy may develop more quickly or be less readily reversed in subjects customarily on a low ascorbic acid intake, and 2) administration of high doses of ascorbic acid might be advantageous in subjects who have been tak-

* Available from Winthrop Laboratories, New York, under the trade name of Aralen®.