pressure. The first question to be resolved is whether puromycin in the dosages used was capable of effectively inhibiting the synthesis of proteins. It would seem that adequate doses were administered; thus puromycin was administered in about 6 times the effective dosages, based on toxicity, of acetoxycycloheximide and actinomycin D. Likewise the dose of 50 mg puromycin per rat, which failed to reduce blood pressure, is 3 to 5 times the amount which other investigators used to demonstrate *in vivo* inhibitory effects on various tissue proteins(6). This failure to lower blood pressure may be related to the mechanism by which puromycin inhibits protein synthesis in inducing release of nascent polypeptides from the ribosomes, rather than suppression of polypeptide synthesis obtained with the other compounds. Whether this feature is coincident with, or has a physiologic significance in blood pressure regulation remains to be demonstrated.

Another illustration of how the specific mechanisms involved in inhibition of protein synthesis may influence circulation may be noted in the difference in blood pressure response to prednisolone between rats treated with actinomycin D and acetoxycycloheximide or chloramphenicol. The reduction of blood pressure by the first compound, which suppresses DNA-dependent RNA synthesis, including m RNA, is not corrected by this steroid. Chloramphenicol and acetoxycycloheximide, on the other hand, which have no effect on m RNA but with the orderly transfer of amino acids to form polypeptides, induce a depressor effect which is opposed by prednisolone. While on the surface it may appear that the presence of m RNA is essential for this hemodynamic response of prednisolone, it is obviously premature to attempt to explain the above results on the basis of available information.

Summary. Acetoxycycloheximide, an inhibitor of protein synthesis at the ribosomes, reduces the blood pressure of normotensive and hypertensive rats. Chloramphenicol, which has a similar but not identical action, also reduces blood pressure of hypertensive rats. The degree of blood pressure depression to both substances is dose-dependent and is appreciable in non-toxic amounts. Puromycin, which inhibits ribosomal synthesis of protein, but through release of rather than suppression of polypeytides, fails to reduce blood pressures at much higher dosage levels relative to toxicity. Blood pressure depression following acetoxycycloheximide or chloramphenicol administration is corrected by prednisolone. Rats treated with acetoxycycloheximide respond to l-norepinephrine with normally vigorous blood pressure elevation suggesting that phasic contractile proteins remain intact.

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Rheumatoid Factor and Immuno-Conglutinin Responses Following Various Vaccinations. (31710)

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Rheumatoid factors exhibit a considerable degree of specificity for rheumatoid arthritis. They can be detected in the great majority of sera from active cases of rheumatoid arth-

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ritis. Positive reactions are also seen in a variety of other disease conditions, especially in infectious diseases and in liver diseases, but less frequently and usually in lower titers than in rheumatoid sera. Rheumatoid factors are encountered only occasionally in the sera of apparently healthy people.

Anti-γ-globulin factors resembling human rheumatoid factors can be produced in experimental animals by hyperimmunization with bacterial(1,2) or well-defined protein(3) antigens. The implication is that these factors are anti-antibodies induced by autologous antibody γG globulin altered in an *in vivo* reaction with the antigen. Rheumatoid factor-like substances can also be produced by immunization with denatured(4,5) or enzymatically degraded(6) autologous γG globulin.

There is good evidence indicating that immuno-conglutinins are autoantibodies directed against some complement component exposed in the complement-fixation reaction (7-9). They can be induced by immunization (autostimulation) with bacterial antigens (10,11), and elevated immuno-conglutinin titers are frequently seen in infectious diseases and in diseases considered to be possibly of autoimmune origin(7).

We have previously presented evidence that the routine vaccinations of army recruits give rise to transient rheumatoid factor reactions (12). The routine vaccinations also were followed by transient false positive serological tests for syphilis. These were confined to the smallpox vaccination, and the role of the other vaccinations seemed not to be significant in this respect(13). The experimentally induced rheumatoid factors and immuno-conglutinins in humans may be useful in understanding the stimulus and significance of these antibodies. It was felt worthwhile, therefore, to study the role of individual vaccinations. In the present communication, evidence is presented suggesting that both the rheumatoid factor and the immuno-conglutinin responses mainly occur after vaccinations other than the smallpox vaccination and that the immuno-conglutinin response occurs earlier than the rheumatoid factor response.

Material and methods. These experiments were performed with 381 healthy military recruits about 20 years of age. Practically all of them had been vaccinated against smallpox in childhood. At the beginning of their service they were all subjected to the following vaccination program: combined tetanus and salmonella vaccine (5 Lf of tetanus toxoid and $10^{8.4}$ *S. typhi*, $10^{7.8}$ *S. paratyphi* B, and $10^{8.1}$ *S. typhi murium*), diphtheria vaccine (1 Lf of toxoid), mumps vaccine (512 hemagglutinating units of formaline-inactivated Enders strain), inactivated polio vaccine (10^{8} TCD_{50} of type I, $10^{7.5}$ of type II and 10^{7} type III), and smallpox vaccination by multiple puncture technic. The smallpox vaccine was the Finnish standard calf lymph vaccine (State Serum Institute, Helsinki), which contained $10^{8.1}$ pock forming units per ml when titrated on chick chorioallantoic membranes, $10^{8.0}$ and $10^{7.4}$ TCD_{50} per ml when assayed in primary human amnion cells and in primary monkey kidney cells, respectively.

Two tests were used to measure the rheumatoid factors. The Waaler-Rose test was performed by technic described in detail elsewhere(14). Sera were first absorbed with sheep cells to remove the heteroagglutinins. For sensitization of the cells, ⅓ of the minimum agglutinating dose of homologous rabbit amboceptor, with the antibodies mainly in the γG globulin fraction, was used. The first serum dilution was 1:16, corresponding to a dilution 1:32 after addition of the sensitized cells. A titer of 64 or higher was regarded as significant. The latex test was performed by the one-tube modification using the patient's own γ-globulin(15). The results were graded as positive, weakly positive and negative.

Immuno-conglutinin titrations were performed by method II A as described by Coombs, Coombs, and Ingram(7). The first serum dilution was 1:4, corresponding to a dilution 1:8 after addition of the alexinated cells.

The blood samples from the same persons were always tested simultaneously. Several controls consisting of weakly positive sera were included in each series to insure as far as possible the same level of sensitivity. All titers are expressed as reciprocals of dilutions

TABLE I. Time Schedules of the Series.

	Day	Vaccinations	Blood sample No.
Previous series (245 recruits)	1	Salmonella, tetanus, mumps and polio	1
	8	Diphtheria and smallpox	
	22–26		2
	43–47		3
Experiment A (189 recruits)	1	Salmonella, tetanus, mumps and polio	1
	15	Diphtheria and smallpox	2
	29		3
Experiment B (192 recruits)	1	Smallpox	1
	15	Salmonella, tetanus, mumps and polio	2
	35	Diphtheria	3

calculated from the total volume of reagents in the tube.

Results. For completeness, the essential results of the previous series(12) are recapitulated and discussed together with the present findings. The exact time schedules of the series appear from Table I.

The results obtained in the rheumatoid factor tests are shown in Table II. There

TABLE II. Rheumatoid Factor Responses.

	No. of positive sera*		
	Sample 1	Sample 2	Sample 3
Previous series	3	13	7
Exp A	6	7	13
Exp B	4	4	9

* Waaler-Rose and/or latex test.

were 13 positive sera in the first blood samples and all remained positive throughout the whole experimental period. This indicates that the positivity in the prevaccination blood samples is a relatively stable characteristic.

In the previous series there were 10 new positive specimens in the second samples and six of these had become negative in the third samples. In Experiment A there was one new positive specimen in the second group of samples and 2 additional positive specimens in the third group of samples. In Experiment B there were no new positive specimens in the second group of samples and 5 new positive specimens in the third.

The majority of the sera showing positive rheumatoid factor reactions (24 out of 35) were positive only in the latex test. One serum was positive only in the Waaler-Rose test. The titers of the Waaler-Rose positive sera ranged from 64 to 512.

Based on Experiments A it seemed possible that the rheumatoid factor responses were related mainly to the smallpox vaccination given 2 weeks prior to the third blood samples. However, not a single positive reaction followed the smallpox vaccination in Experiment B. The most reasonable explanation is that the rheumatoid factor responses were related to the first vaccinations (salmonella, tetanus, mumps and polio) but that it took longer than two weeks before the factors could be detected in the serum. It was not possible so to adjust the vaccinations and to take blood samples as to prove definitely that this was the case.

Immuno-conglutinin responses were studied in Experiment A. The results are compiled in Table III. It is apparent that there is in many persons a rapid increase of immuno-conglutinin titers, and the titers were declining at the time when the third blood samples were taken. There was no correlation with the initially high immuno-conglutinin titers

TABLE III. Immuno-Conglutinin Responses.

	Sample 1	Sample 2	Sample 3
Average immuno-conglutinin titer	3.5	7.6	5.1
No. of sera with titer $\geqq 32$	6	17	8
No. of paired sera with significant* titer increase	—	21	1
No. of paired sera with significant* titer reduction	—	0	5

* 4-fold or over.

and the rheumatoid factor reactions. None of the 22 persons with significant immuno-conglutinin titer increases showed either persistent or induced rheumatoid factor reactions.

Discussion. Several possible bases, not mutually exclusive, can be considered to explain the frequent occurrence of rheumatoid factors in high titers in the sera of patients with rheumatoid arthritis: a) a genetic predisposition of patients with rheumatoid arthritis to produce autoreactive anti-γ-globulin factors; b) a hitherto unknown very efficient antigenic stimulus, possibly of viral origin, that maintains a hyperimmune state with a continuing *in vivo* formation of antigen/antibody complexes; and c) specific conditions in the diseased joints and other organs that lead to enzymatic degradation or denaturation of γG globulin.

In a previous study(13), false positive serological tests for syphilis following the army vaccinations were studied. It was shown that they were confined to the smallpox vaccination, whereas the role of the other vaccinations was insignificant in this respect. Evidence was presented that multiplication of the vaccinia virus was necessary for the false positive reactions to occur, and the reactions probably were related to the tissue injury caused by the living virus. The results of the present series as a whole hardly lend support to the thesis that smallpox vaccination plays a major role in the formation of rheumatoid factors although Experiment A alone seemed to suggest so. Rather, it seems plausible that both the rheumatoid factor and the immuno-conglutinin responses somehow are related to the net immune response provoked by the vaccinations.

Two points were noted that differentiated the rheumatoid factor and the immuno-conglutinin responses from each other. First, the immuno-conglutinin response occurred earlier. The immuno-conglutinin titers were declining at the time when detectable amounts of rheumatoid factors appeared in the circulation. To our knowledge no information of simultaneous follow-up studies of the appearance of rheumatoid factor-like substances and immuno-conglutinins in experimental animals has been published. However, the time relationships appearing from separate studies of the induction of rheumatoid factor-like substances(1,2) and of immuno-conglutinins(10, 11) by immunization with bacterial antigens seem to suggest that the same holds true for experimental systems. These time relationships suggest that the basic mode of genesis of rheumatoid factors and of immuno-conglutinins and/or their physiological function, if any, may differ from each other.

The second point is that the rheumatoid factor and immuno-conglutinin responses were not encountered in the same persons. It is possible that the recruits with positive rheumatoid factor reactions are potential victims for rheumatoid arthritis. Thus this observation may be related to the circumstance that elevated immuno-conglutinin titers are seen in many disease conditions where immunological events are known or suspected to play a role(7), whereas the rheumatoid factors show a considerable degree of specificity for rheumatoid arthritis.

Summary. Rheumatoid factor and immuno-conglutinin responses following various vaccinations were studied in a series of 381 healthy military recruits. The results suggested that both types of reaction were related to the net immune response provoked by the vaccinations. The immuno-conglutinin response took place within 2 weeks, whereas 3-4 weeks were needed for the appearance of rheumatoid factors. Immuno-conglutinin titer increases and positive rheumatoid factor reactions were not seen in the same persons.

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Imidazole Lactic Acid—A Component of Normal Human Urine.* (31711)

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In Europe, the only diazo-positive spot found after paper chromatography of 20 μl of urine is histidine(1); other spots would be considered abnormal. Recently a laboratory was established here for screening of non-human primate blood and urine by a variety of chromatographic and electrophoretic techniques, and the initial program included the training of technicians using their own urine and blood specimens. Following the application of a histidine-locating reagent to a paper chromatogram of urine specimens, one of the two examined showed a second red spot as well as a number of other red, gold and yellow spots. Subsequently 30 urines were obtained from laboratory staff and others and varying quantities of the second red spot were found in many but not all chromatogrammed specimens.

Materials and methods. Random urine specimens were obtained from 30 white and colored adults and infants; serum was obtained from all of the adults. All urines were desalted electrolytically(1) and one of the same urines was also desalted by ion-exchange. Urines were chromatographed on paper(1) and on thin layers of Avicel(2) but serum was run on paper(3) only. Butanol-acetic acid-water was used as the standard one-way solvent but other solvents and two-way procedures were also run to confirm identity of imidazoles. The DNPH-electrolytic reduction method(1) was carried out on a number of specimens to investigate the presence of imidazole pyruvic acid. Ethyl acetate extractions were made to distinguish between imidazoles which would be insoluble and phenolic acids and indoles, etc., which would be soluble in the reagent.

Results. A number of red and red-brown spots were found on urine chromatograms. The main component was histidine, with variable amounts of imidazole lactic acid and traces of other imidazoles. Parallel and co-chromatography with imidazole lactic acid confirmed the nature of the second imidazole compound. One of the trace components appeared to be imidazole propionic acid, although as the samples had been electrolytically desalted it might have been derived from urocanic acid; serum specimens showed none of the above compounds with occasional exceptions of traces of histidine.

Discussion. As judged from the spot area and intensity of color, the imidazole lactic acid spot was present in considerable although variable amounts in relation to the histidine concentration in a cross-section of the local population. A parallel study of over 200 non-human primates (to be reported later) of a variety of species including marmosets, baboons, capuchin monkeys, etc., showed a variable number of imidazoles to be present in the urine. Blood specimens from all species showed little or no histidine and no other spots when 8-10 μl were examined al-

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