by the microscopic examination, with no morphological changes attributable to the subtilin being observed. Tissues studied in this manner included liver, kidney, spleen, pancreas, adrenal, testis, heart, lung and thyroid.

Conclusions. The feeding to rats of diets containing as high as 0.1% of subtilin for 220 days was not detrimental as judged by growth, activity, organ weights and macroscopic and microscopic appearance of the tissues. The previously reported evidence for precipitation of parenterally-administered subtilin has been substantiated by microscopic examination.

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Hemagglutination Reaction in Streptococcal Infections and Acute Rheumatic Fever.* (19125)

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A hemagglutination test described by Middlebrook and Dubos(1) is being widely studied in tuberculous infections in experimental animals and in man. Preliminary evidence indicates that this reaction has greater specificity than other serological procedures such as the complement fixation test, and that it tends to be positive only during active tuberculous infections(1,2).

These promising results suggested the possibility that hitherto undetected antibodies might be discovered during the active phase of rheumatic fever with the hemagglutination technic. The present study was undertaken, therefore, to determine whether various streptococcal extracts or products could be adsorbed onto sheep erythrocytes, and to observe the behavior of the sensitized cells in the presence of sera from patients with streptococcal infections and acute rheumatic fever.

Materials and methods. The following streptococcal filtrates and extracts were prepared for hemagglutination tests: A. *Heated filtrate of a culture of whole streptococci.* A Group A, non-typable streptococcus recently isolated from a patient with acute rheumatic fever was grown overnight in 500 cc of Todd-Hewitt broth. The culture was placed in a boiling water bath, and the total volume was reduced from 500 cc to 100 cc by evaporation. Following Seitz filtration, the filtrate was diluted 1 to 6 in normal saline for attempts at sensitization of sheep erythrocytes. When negative results were obtained, the filtrate was further concentrated from 100 cc to 20 cc by evaporation. Even when diluted 1 to 6 this thick, syrupy material was unsatisfactory for sensitization because it caused hemolysis of most of the red cells. B. *Hydrochloric acid extract.* The streptococcus used was the same as for preparation A. The crude streptococcal extract, containing both the group-specific C substance and the type-specific M antigen was prepared in the usual fashion(3) by boiling the organisms with HCl at a pH of 2.5, discarding the cellular residue, and neutralizing the supernatant. The extract was diluted 1 to 4 in normal saline for attempts at sensitization. C. *Formamide extract.* The method of Fuller(4) was used to extract the C substance. The streptococcus was the same as that used for preparations A and B. For sensitization the extract was diluted 1 to 4 in saline. D. *Streptolysin O.* Streptolysin O was prepared from the Richards strain according to the method of Rantz and Randall(5), and was stored in the refrigerator in the oxidized state.

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TABLE I. Antistreptolysin and Hemagglutination Titers of 8 Patients with Non-Streptococcal Upper Respiratory Infections, and of 7 Patients with Acute Rheumatic Fever.

	Patient	Antistreptolysin titer (units/cc)	Sensitizing antigen: Formamide extr.	Sensitizing antigen: Streptolysin O
Patients with acute non-streptococcal respiratory infections	1	50	1:4	1:8
	2	<12	1:64	1:64
	3	12	1:4	1:4
	4	100	0	0
	5	50	1:128	1:128
	6	66	1:64	1:32
	7	250	0	0
	8	12	1:32	1:16
Patients with acute rheumatic fever	1	833	1:32	1:32
	2	1250	1:16	1:32
	3	625	1:4	1:4
	4	1250	Not done	1:128
	5	833	" "	1:8
	6	625	" "	1:8
	7	833	" "	1:64

It was diluted 1 to 6 in saline for sensitization.

Method of performing hemagglutination tests. The modification of Smith and Scott (6), which differs only slightly from the original technic of Middlebrook and Dubos, was used in the present studies. Briefly, sheep erythrocytes were suspended in the sensitizing antigen solution for two hours at 37°C, washed gently, and resuspended in saline. Agglutination tests were performed by adding the sensitized cells to serial dilutions of heat-inactivated patients' sera. The tubes were incubated at 37°C for 2 hours, and allowed to stand overnight at room temperature. Agglutination was detected by gently shaking the tubes the morning after tests were performed. Tubes with definite macroscopic clumps were called positive; those with an abnormal cell pattern on the bottom of the tube, but without definite clumping, were called negative.

Results. Preparations A and B. Sensitization of sheep red cells did not occur with these two preparations; *i.e.*, there was no agglutination of the treated cells by sera which gave definite agglutination with preparations C and D. *Preparations C and D.* With both of these extracts there was sensitization of sheep erythrocytes with clear-cut hemagglutination in some specimens of human sera. Hemagglutination tests were performed with sera from patients with acute non-streptococcal upper respiratory infections, probably viral in etiology, and from patients with acute rheumatic fever. Representative results are presented in Table I. The hemagglutination titers were identical with the two sensitizing antigens, and there was no correlation between the antistreptolysin titers and the dilutions of sera which caused agglutination of the sensitized red cells. Finally, the hemagglutination titers recorded in patients with acute rheumatic fever fell in the same range as those observed in patients with non-streptococcal respiratory infections.

An attempt was then made to differentiate individuals with acute streptococcal sore throats from patients with acute rheumatic fever on the basis of the hemagglutination reaction. Acute and convalescent sera were obtained from 11 young adults with streptococcal sore throats. Five developed acute rheumatic fever before the second blood specimen was drawn. These sera were obtained through the courtesy of Dr. Charles H. Rammelkamp, Director of the Streptococcal Disease Laboratory, Warren Air Force Base, Wyoming. Code numbers were used for the sera, so that the results could not be influenced by knowledge of the clinical picture.

The results are presented in Table II. Definite hemagglutination was observed with all but one of the sera tested, but again there

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TABLE II. Hemagglutination Titers of Sera Obtained from 11 Young Adults with Acute Streptococcal Pharyngitis. Five developed acute rheumatic fever before the convalescent blood was drawn.

	Patient	Type of infecting streptococcus	Antistreptolysin titer (units/cc)		Hemagglutination titers	
			Acute sera	Convalescent sera	Acute sera	Convalescent sera
Patients with acute streptococcal pharyngitis who did not develop rheumatic fever	1	28	250	400	1:8	1:4
	2	14	83	250	1:16	1:32
	3	14	50	400	Not done	1:16
	4	1	62	125	1:128	1:128
	5	14	62	250	1:64	1:128
	6	14	50	317	Not done	1:16
Patients who developed acute rheumatic fever	1	14	100	317	1:16	1:16
	2	14	83	833	1:4	1:8
	3	14	125	317	1:32	1:32
	4	14	125	200	1:64	1:128
	5	14	<50	317	0	Not done

was a complete lack of correlation between the antistreptolysin and hemagglutination titers. Patients with acute rheumatic fever could not be differentiated from those with streptococcal pharyngitis on the basis of the hemagglutination test. The one consistent observation was that, for any one patient, the titers tended to be the same for both the acute and convalescent specimens.

Discussion. The present study demonstrates that various streptococcal products can be adsorbed onto sheep red blood cells, and that erythrocytes so sensitized are agglutinated by a variety of human sera. It was rather surprising to find identical hemagglutination titers with the formamide extract, which is a relatively pure preparation of the group-specific carbohydrate (C substance), and concentrated streptolysin O, which is a protein. The exact nature of the sensitizing material cannot be determined from these data, but the evidence suggests that it is the carbohydrate, with a sufficient amount of the C substance being present in the streptolysin solution to sensitize erythrocytes as effectively as the antigen in the formamide extract.

The failure of the other two preparations (A and B) to sensitize red cells is not entirely clear. The evaporated culture supernatant of intact streptococcal bodies (a preparation similar to Old Tuberculin) may simply have been less concentrated than the ammonium sulfate precipitated preparation of streptolysin O. The failure of the acid extract to sensitize cells, in contrast to the formamide extract which gave excellent adsorption, is less easily understood. It is possible that the presence of the M protein interfered in some way with adsorption of the C substance.

With human sera, clear-cut hemagglutination was present in titers as high as 1:128 with preparations C and D. There was a complete lack of correlation between hemagglutination and antistreptolysin titers, and between hemagglutination titers and the presence or absence of streptococcal infections or rheumatic fever. The one consistent finding was that the hemagglutination titers tended to be the same during the acute and convalescent stages of streptococcal infections (Table II). This suggests that the substance in the serum which is responsible for hemagglutination may have nothing to do with infection by the streptococcus, either past or present. If the serum substance is a true antibody, it may be present as a result of stimulation by an agent possessing antigenic constituents identical with certain components of the Group A streptococcus. Further study will be necessary to elucidate the exact nature of the phenomenon.

Summary and conclusions. (1) The hemagglutination technic of Middlebrook and Dubos has been applied to the study of streptococcal infections and rheumatic fever. Two of 4 streptococcal extracts gave excellent sensitization of sheep erythrocytes. These were the formamide extract, containing the group specific carbohydrate, and a concentrated solu-

tion of streptolysin O, which is a protein. Surprisingly, hemagglutination titers were identical with a variety of human sera when these two preparations were used as sensitizing antigens. (2) Hemagglutination was shown by many sera in titers ranging from 1:4 to 1:128, but there was no correlation between the magnitude of antistreptolysin and hemagglutination titers. Patients with non-streptococcal respiratory infections, streptococcal pharyngitis, and acute rheumatic fever could not be differentiated on the basis of the hemagglutination test. (3) The one consistent observation was that, for any one patient, hemagglutination titers tended to remain constant with both acute and convalescent sera. This suggests that a cross reaction of some type was responsible for the agglutination of red cells sensitized with streptococcal products. Further study is necessary to clarify the nature of this phenomenon.

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B Vitamin Content of Rat Adrenals with Respect to Exposure to Cold. (19126)

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Sayers(5) has ably reviewed the biochemical changes observed in the adrenal gland under stress. While it has been noted that stress stimuli effect the depletion of ascorbic acid(6) and cholesterol(7) and the turnover of phosphorus(2) in the adrenal cortex, no data are available to indicate whether other substances, particularly other vitamins, are similarly affected. There is presented here the results of a study of the comparative concentration of four B-vitamins (biotin, folic acid, niacin and riboflavin) in the adrenals of rats before and after subjection to cold stress for one hour at 5°C.

Materials and methods. Female rats of the Holtzman strain, weighing approximately 150 g, were used. All had access to a stock diet of N.C.I. food pellets(3) and tap water *ad libitum*, supplemented weekly with 0.2 ml administered cod liver oil. Twenty rats constituted each experimental group. Ascorbic acid was determined by the method of Roe and Kuether(4) as described by Sayers(8) with modifications in temperature and time of incubation as outlined in the procedure below. The B-vitamins were liberated enzymatically, as recommended by Cheldelin(1), with papain and takadiastase. They were assayed microbiologically with *L. casei* in 2 ml volume, employing a medium essentially similar to that described by Tepley and Elvehjem(9) with appropriate supplements and omissions for adaptation to the several vitamin assays. The rats were anesthetized with ether, the left adrenals were removed and weighed to the nearest milligram and dropped immediately into McShan homogenizers containing one ml

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