the intestines. Difco Levine EMB agar, prepared both with fresh and with sea water, was used as the isolating medium. The medium was poured into plates and streaked with the samples from the sea lion.

After 24 hr incubation, at both 27° and 37° C, the EMB plates were examined, and the presence of coliform organisms was established in both the lower end of the small intestine and in the entire large intestine. The stomach appeared to be sterile. Typical coliform organisms appeared on the sea-water medium, but the colonies on fresh-water medium lacked their differentiating metallic sheen. All other features, including routine differential media, indicated that the organisms were *E. coli*. The isolated *E. coli* cultures grew as well, at either 27° or 37° C, on sea-water as they did on fresh-water medium; hence these organisms may have been indigenous to the sea or were more resistant forms from terrestrial contamination. *E. coli* will normally tolerate limited exposure to sea water, but the conventionally known strains are reportedly either killed quantitatively or diluted to virtual extinction by sea water in full strength (*2*).

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Autohemagglutinins in the Serum of Patients with Acute Lupus Erythematosus

A RECENT note by Schleicher (*1*) described a test for acute lupus erythematosus, in which Group O Rh-positive erythrocytes were agglutinated by the patient's serum in a saline solution of egg albumin. The agglutination disappeared at room temperature and at 37° C but reappeared at refrigerator temperatures.

Fjelde (*2*), using a modification of Schleicher's procedure, has reported detecting the erythrocyte aggregation factor in the serum of 17 out of 17 patients with the acute form of lupus erythematosus. The factor was not identified.

The similarity of the tests used by Schleicher and Fjelde to those usually employed to detect autohemagglutinins in the serum of patients presenting primary atypical pneumonia syndromes, hemolytic syndromes, and occasional other disorders (*3*) prompted us to investigate the matter further. In addition, we had previously noted that autohemagglutinins active at refrigerator temperatures were frequently present in the serum of patients suffering from acute lupus erythematosus.

Samples of blood were obtained from 7 patients with acute disseminated lupus erythematosus, 2 with noncongenital hemolytic syndromes, 3 with miscellaneous disorders—including 1 patient with pneumonitis presumed to be of viral origin—and 20 persons whose serum did not contain autohemagglutinins.

The method used in our laboratory seldom gives positive results with serum from normal persons; it consists of serial dilutions of serum in 0.9% sodium chloride solution, ranging from 1:4 through 1:2048, to which is added 0.1 ml 2% suspension of washed O Rh-positive erythrocytes. The tubes are refrigerated overnight and examined for the presence of agglutination. The foregoing test was performed on samples of serum from all the cases. Venous blood was allowed to clot at room temperature, and the serum was removed and used at once, or was frozen for use in the near future.

The method described by Schleicher was also used on duplicate samples and at the same time. To 1 ml refrigerated 10% egg albumin in 0.9% sodium chloride solution, 0.5 ml serum was added, and the fluids were mixed. One drop (0.05 ml) of a 10% suspen-

TABLE 1
RESULTS OF TESTS

Case	Clinical diagnosis	Cold agglutinin titer	Schleicher test	Schleicher test after absorption with O Rh + erythrocytes in the cold
1	Acute lupus erythematosus with hemolytic anemia	1:8	+	
2	" " " " " "	1:32	+	Negative
3	Subacute lupus erythematosus	Negative	Negative	
4	" " "	"	"	
5	Acute lupus erythematosus	1:8	+	"
6	" " "	1:16	+	"
7	" " "	1:16	+	"
8	Noncongenital hemolytic syndrome	1:8	+	
9	" " "	1:32	+	"
10	Hyperglobulinemia with multiple serologic abnormalities	1:8	+	"
11	Acute leukemia with indeterminate lesion in lung	1:16	+	
12	Pneumonitis	1:32	+	
13–32	Normal controls	Negative	Negative	

sion of washed cold O Rh-positive erythrocytes was added to each tube. The tubes were kept at refrigerator temperature for 30 min, centrifuged at 1000 rpm for 1 min, and examined for agglutination. Besides the negative serum controls, we found it useful to prepare an albumin solution control of 1 ml albumin solution, 0.5 ml saline, and 0.05 ml of the cell suspension. It was found that the albumin solution could be frozen in small amounts, thus obviating the necessity of preparing a fresh solution the day before each test. It was also found that a 10% suspension of O Rh-positive cells could be prepared the day of the test, if the final dilution was made up in refrigerated saline.

All the serums that gave positive results with a titer of 1:8 or greater with our standard "cold agglutination" test also gave strongly positive results with the Schleicher method. These included 5 of the 7 cases of acute lupus erythematosus. All the serums that gave negative results with our standard agglutination tests also gave negative results with the Schleicher method (Table 1).

Absorption tests were performed on most of the positive serums in order to identify the erythrocyte aggregation factor of Schleicher and Fjelde as a cold agglutinin. The tests were performed by adding 25% by volume of packed washed O Rh-positive erythrocytes to samples of serum and allowing the mixtures to stand for 4 hr in the refrigerator. The supernatant serum was then removed, and both standard and Schleicher tests were performed on the absorbed serum and on corresponding untreated serums that had been allowed to stand simultaneously in the refrigerator. The absorbed serums all gave negative results with both tests, whereas their corresponding serum controls gave positive results by both methods. Negative controls were included with all tests.

Additional evidence that the autohemagglutinin detected by the standard method is similar to that shown by the albumin method was found when some of the positive serums were retested after several weeks in the frozen state. The results were commonly weak or negative. It is known that cold agglutinins often decrease in titer with storage, even when frozen.

We conclude that the aggregation factor in the serum of patients with acute lupus erythematosus described by Schleicher and Fjelde is similar to, or identical with, the cold agglutinin frequently observed in patients with primary atypical pneumonia syndromes, acute hemolytic syndromes, and certain other conditions.

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Microbiological Conversion of Pregnenolone to Progesterone

THE conversion of pregnenolone to progesterone by the bacterium *Corynebacterium mediolanum* has been reported by Mamoli (*Ber.*, **71**, 2701 [1938]). More recently the same oxidation has been observed in liver slices and other tissues by Samuels *et al.* (SCIENCE, **113**, 390 [1951]). We have found that other microorganisms, including actinomycetes and molds, are capable of carrying out this oxidation. In our studies, a group of species of streptomyces, including *Streptomyces griseus, S. fradiae, S. aureofaciens,* and *S. rimosus,* were grown in submerged aerated culture (100 ml medium/500-ml flask) on a soybean meal–glucose-soybean oil medium, to which was added 20 mg/100 ml of pregnenolone. After 24 hr incubation the fermentations were extracted thrice with an equal volume of chloroform. The extracts were pooled, and aliquots examined by Zaffaroni's filter-paper partition chromatographic technique (SCIENCE, **111**, 6 [1950]), using the toluene-propylene glycol system. The steroid located on one of a number of replicate strips using the Zimmerman reagent, following a 1-hr development period, had a mobility equal to that of a progesterone standard. The steriod-containing areas of other strips were eluted with ethanol, pooled, and identified by the procedures used by Samuels *et al.* (*loc. cit.*)—i.e., measurement of absorption spectrum of the steroid and of its dinitrophenylhydrazone derivative. The steroid in these ethanol eluates had an absorption maximum at 240 mμ, which might be explained by oxidation of the 3-hydroxyl group to a keto group, accompanied by a shift of the 5, 6 double bond to the 4, 5 position. Pregnenolone has no absorption maximum at 240 mμ. The dinitrophenylhydrazone derivative of the steroid in the ethanol eluates had an absorption spectrum similar to that obtained with progesterone bisdinitrophenylhydrazone. These data, together with the mobility in the filter-paper chromatographic system and the absorption spectrum of the steroid, suggest that progesterone was present in the chloroform extracts of the fermentations.

A similar series of fermentations was carried out, using *Phycomyces blakesleeanus, Aspergillus niger, Penicillium chrysogenum, Eremothecium ashbyii,* and *Ustilago zeae* cultures grown in submerged aerated culture on a cornsteep liquor–calcium carbonate–glucose-soybean oil medium supplemented with pregnenolone at a level of 20 mg/100 ml. The steroid recovered in the chloroform extracts of these cultures had the same characteristics as those found in the extracts of the actinomycete cultures. These experiments indicate that actinomycetes and molds are able to carry out the biological oxidation of pregnenolone to progesterone, first observed by Mamoli, who used bacteria, and more recently by Samuels *et al.*, who used liver and other tissue preparations.

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